

Title of the Invention:**Tri-Specific Binding Molecules That Specifically Bind to Multiple Cancer Antigens and Methods of Use Thereof****Cross-Reference to Related Applications**

[0001] This application claims priority to United States Patent Applications No. 62/107,824 (filed January 26, 2015), 62/008,229 (filed June 5, 2014; pending), and 62/004,571 (filed May 29, 2014; pending), each of which applications is herein incorporated by reference in its entirety.

Reference to Sequence Listing:

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in computer-readable media (file name: 1301_0119PCT_Sequence_Listing_ST25.txt, created on 18 May 2015, and having a size of 416,408 bytes), which file is herein incorporated by reference in its entirety.

Background of the Invention:**Field of the Invention:**

[0003] The present invention relates to Tri-Specific Binding Molecules, which are multi-chain polypeptide molecules that possess three Binding Domains and are thus capable of mediating coordinated binding to three epitopes. The Tri-Specific Binding Molecule is preferably characterized in possessing binding domains that permit it to immunospecifically bind to: (1) an epitope of a first Cancer Antigen, (2) an epitope of a second Cancer Antigen, and (3) an epitope of a molecule that is expressed on the surface of an immune system effector cell, and are thus capable of localizing an immune system effector cell to a cell that expresses a Cancer Antigen, so as to thereby facilitate the killing of such cancer cell.

Description of Related Art:**I. The Mammalian Immune System**

[0004] The mammalian immune system serves as a defense against a variety of conditions, including, *e.g.*, injury, infection and neoplasia. The efficiency with which humans and other mammals develop an immunological response to pathogens, foreign substances and cancer antigens rests on two characteristics: the exquisite specificity of the immune response for antigen recognition, and the immunological memory that allows for faster and more vigorous responses upon re-activation with the same antigen (Portolés, P. *et al.* (2009) “*The TCR/CD3 Complex: Opening the Gate to Successful Vaccination*,” *Current Pharmaceutical Design* 15:3290-3300; Guy, C.S. *et al.* (2009) “*Organization of Proximal Signal Initiation at the TCR:CD3 Complex*,” *Immunol Rev.* 232(1):7-21).

[0005] The mammalian immune system is mediated by two separate but interrelated systems: the cellular and humoral immune systems. Generally speaking, the humoral system is mediated by soluble products (antibodies or immunoglobulins) that have the ability to combine with and neutralize products recognized by the system as being foreign to the body. In contrast, the cellular immune system involves the mobilization of certain cells, termed “T cells,” that serve a variety of therapeutic roles. T cells are lymphocytes that are derived from the thymus and circulate between the tissues, lymphatic system and the circulatory system. In response to the presence and recognition of foreign structures (antigens), T cells become “**activated**” to initiate an immune response. In many instances these foreign antigens are expressed on host cells as a result of neoplasia or infection. Although T cells do not themselves secrete antibodies, they are usually required for antibody secretion by the second class of lymphocytes, B cells (which derive from bone marrow). Critically, T cells exhibit extraordinary immunological specificity so as to be capable of discerning one antigen from another). Two types of T cells, “T helper cells” and “cytotoxic T cells,” are of particular relevance.

[0006] T helper cells are characterized by their expression of the glycoprotein, **CD4** (*i.e.*, they are “CD4⁺”). CD4⁺ T cells are the essential organizers of most mammalian immune and autoimmune responses (Dong, C. *et al.* (2003) “*Immune*

Regulation by Novel Costimulatory Molecules,” Immunolog. Res. 28(1):39-48). The activation of CD4⁺ T cells has been found to be mediated through co-stimulatory interactions between an antigen:major histocompatibility class II (**MHC II**) molecule complex that is arrayed on the surface of an Antigen Presenting Cell (such as a B cell, a macrophage or a dendritic cell) and a complex of two molecules, the T Cell Receptor (“**TCR**”) and a **CD3** cell surface receptor ligand, that are arrayed on surface of a naive CD4⁺ T cell. Activated T helper cells are capable of proliferating into Th1 cells that are capable of mediating an inflammatory response to the target cell.

[0007] Cytotoxic T cells are characterized by their expression of **CD8** (i.e., they are “CD8⁺” as well as CD3⁺). The activation of CD8⁺ T cells has been found to be mediated through co-stimulatory interactions between an antigen:major histocompatibility class I (**MHC I**) molecule complex that is arrayed on the surface of a target cell and a complex of CD8 and the T Cell Receptor, that are arrayed on surface of the CD8⁺ T cell. Unlike MHC II molecules, which are expressed by only certain immune system cells, MHC I molecules are very widely expressed. Thus, cytotoxic T cells are capable of binding to a wide variety of cell types. Activated cytotoxic T cells mediate cell killing through their release of the cytotoxins perforin, granzymes, and granulysin. Through the action of perforin, granzymes enter the cytoplasm of the target cell and their serine protease function triggers the caspase cascade, which is a series of cysteine proteases that eventually lead to apoptosis (programmed cell death) of targeted cells.

[0008] The T cell receptor (“**TCR**”) is a covalently linked heterodimer of α and β chains (“**TCR $\alpha\beta$** ”). These chains are class I membrane polypeptides of 259 (α) and 296 (β) amino acids in length. The **CD3** molecule is a T cell co-receptor composed of five distinct polypeptide chains (a **CD3 γ** chain, a **CD3 δ** chain, two **CD3 ϵ** chains and two zeta chains). The individual polypeptide chains associate to form a complex of three dimers ($\epsilon\gamma$, $\epsilon\delta$, $\zeta\zeta$) (Wucherpfennig, K.W. *et al.* (2010) “*Structural Biology Of The T Cell Receptor: Insights into Receptor Assembly, Ligand Recognition, And Initiation of Signaling*,” Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14; Chetty, R. *et al.* (1994) “*CD3: Structure, Function And The Role Of Immunostaining In Clinical Practice*,” J. Pathol. 173:303-307; Guy, C.S. *et al.* (2009) “*Organization*

of Proximal Signal Initiation at the TCR:CD3 Complex,” Immunol Rev. 232(1):7-21; Call, M.E. *et al.* (2007) “*Common Themes In The Assembly And Architecture Of Activating Immune Receptors*,” Nat. Rev. Immunol. 7:841-850; Weiss, A. (1993) “*T Cell Antigen Receptor Signal Transduction: A Tale Of Tails And Cytoplasmic Protein-Tyrosine Kinases*,” Cell 73:209-212). The CD3 complex associates with TCR in order to generate an activation signal in T lymphocytes. In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T Cell Receptor Gene Transfer*,” Immunology 129(2):170–177). CD3 is found bound to the membranes of all mature T cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE,” 6th ed. Garland Science Publishing, NY, pp. 214-216; Sun, Z. J. *et al.* (2001) “*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The CD3ε:γ Heterodimer*,” Cell 105(7):913-923; Kuhns, M.S. *et al.* (2006) “*Deconstructing The Form And Function Of The TCR/CD3 Complex*,” Immunity. 2006 Feb;24(2):133-139).

[0009] The TCR and CD3 complex, along with the CD3 ζ chain zeta chain (also known as T cell receptor T3 zeta chain or CD247) comprise the TCR complex (van der Merwe, P.A. *etc.* (epub Dec. 3, 2010) “*Mechanisms For T Cell Receptor Triggering*,” Nat. Rev. Immunol. 11:47-55; Wucherpfennig, K.W. *et al.* (2010) “*Structural Biology of the T cell Receptor: Insights into Receptor Assembly, Ligand Recognition, and Initiation of Signaling*,” Cold Spring Harb. Perspect. Biol. 2:a005140). The complex is particularly significant since it contains a large number (ten) of immunoreceptor tyrosine-based activation motifs (ITAMs).

[0010] Two interactions are required for T cell activation (Viglietta, V. *et al.* (2007) “*Modulating Co-Stimulation*,” Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) “*Checkpoint Blockade in Cancer Immunotherapy*,” Adv. Immunol. 90:297-339). In the first interaction, a Cell must display the relevant target antigen bound to the cell’s major histocompatibility complex so that it can bind to the T cell Receptor (“TCR”) of a naive T lymphocyte. In the second interaction, a ligand of the Cell must bind to a co-receptor of the T lymphocyte (Dong, C. *et al.* (2003) “*Immune*

Regulation by Novel Costimulatory Molecules,” Immunolog. Res. 28(1):39-48; Lindley, P.S. et al. (2009) “The Clinical Utility Of Inhibiting CD28-Mediated Costimulation,” Immunol. Rev. 229:307-321). T cells experiencing both stimulatory signals are then capable of responding to cytokines (such as Interleukin-2 and Interleukin-12). In the absence of both co-stimulatory signals during TCR engagement, T cells enter a functionally unresponsive state, referred to as clonal anergy (Khawli, L.A. et al. (2008) “Cytokine, Chemokine, and Co-Stimulatory Fusion Proteins for the Immunotherapy of Solid Tumors,” Exper. Pharmacol. 181:291-328). In pathologic states, T cells are the key players of various organ-specific autoimmune diseases, such as type I diabetes, rheumatoid arthritis, and multiple sclerosis (Dong, C. et al. (2003) “Immune Regulation by Novel Costimulatory Molecules,” Immunolog. Res. 28(1):39-48).

[0011] The need for two signals to activate T cells such that they achieve an adaptive immune response is believed to provide a mechanism for avoiding responses to self-antigens that may be present on an Antigen Presenting Cell at locations in the system where it can be recognized by a T cell. Where contact of a T cell with a Cell results in the generation of only one of two required signals, the T cell does not become activated and an adaptive immune response does not occur.

II. Antibodies and Other Epitope-Binding Molecules

A. Antibodies

[0012] “Antibodies” are immunoglobulin molecules capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Domain of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, camelized antibodies, single-chain antibodies, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id and anti-anti-Id antibodies to antibodies of the invention), but also mutants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen recognition site of the required specificity, humanized antibodies, and chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. Throughout this

application, the numbering of amino acid residues of the light and heavy chains of antibodies is according to the EU index as in Kabat *et al.* (1992) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242. As used herein, an “antigen-binding fragment of an antibody” is a portion of an antibody that possesses at least one antigen recognition site. As used herein, the term encompasses fragments (*e.g.*, Fab, Fab', F(ab')₂ Fv), disulfide-linked bispecific Fvs (sdFv), intrabodies, and single-chain molecules (*e.g.*, scFv). In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0013] Natural antibodies (such as IgG antibodies) are composed of two **Light Chains** complexed with two **Heavy Chains**. Each Light Chain contains a Variable Domain (**VL**) and a Constant Domain (**CL**). Each heavy chain contains a Variable Domain (**VH**), three Constant Domains (**CH1**, **CH2** and **CH3**), and a Hinge Domain located between the **CH1** and **CH2** Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal (“N”) portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal (“C”) portion of each chain defines a constant region, with light chains having a single Constant Domain and heavy chains usually having three Constant Domains and a hinge region. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is n-VH-CH1-H-CH2-CH3-c (where H is the hinge region, and n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide).

[0014] The ability of an intact, unmodified antibody (*e.g.*, an IgG antibody) to bind an epitope of an antigen depends upon the presence of Variable Domains on the immunoglobulin light and heavy chains (*i.e.*, the VL Domain and VH Domain, respectively). Interaction of an antibody Light Chain and an antibody heavy chain and, in particular, interaction of its VL and VH Domains forms one of the epitope-

binding sites of the antibody. The variable regions of an IgG molecule consist of the complementarity determining regions (**CDR**), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments (**FR**), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the VL and VH Domains have the structure n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c. Polypeptides that are (or may serve as) the first, second and third CDR of an antibody Light Chain are herein respectively designated **CDR_L1 Domain**, **CDR_L2 Domain**, and **CDR_L3 Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of an antibody heavy chain are herein respectively designated **CDR_H1 Domain**, **CDR_H2 Domain**, and **CDR_H3 Domain**. Thus, the terms CDR_L1 Domain, CDR_L2 Domain, CDR_L3 Domain, CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to an specific epitope regardless of whether such protein is an antibody having light and heavy chains or a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein. In contrast to such antibodies, the scFv construct comprises a VL and VH Domain of an antibody contained in a single polypeptide chain wherein the Domains are separated by a flexible linker of sufficient length to allow self-assembly of the two Domains into a functional epitope-binding site. Where self-assembly of the VL and VH Domains is rendered impossible due to a linker of insufficient length (less than about 12 amino acid residues), two of the scFv constructs may interact with one another to form a bivalent molecule in which the VL of one chain associates with the VH of the other (reviewed in Marvin *et al.* (2005) "*Recombinant Approaches To IgG-Like Bispecific Antibodies*," Acta Pharmacol. Sin. 26:649-658).

[0015] In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) "*The Use Of Antibodies In The Treatment Of Infectious Diseases*," Singapore Med. J. 50(7):663-666). Nearly 200 antibody-based drugs have been approved for use or are under development.

[0016] The term “**monoclonal antibody**” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂ Fv), single-chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of “antibody.” Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) “*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*,” *Nature* 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) “*Review of Selected Adjuvants Used in Antibody Production*,” *ILAR J.* 37(3):119-125).

[0017] In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or

may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate a chimeric antibody, a humanized antibody, or a caninized antibody, or to improve the affinity, or other characteristics of the antibody. The term “**humanized**” antibody refer to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and /or sequence of a human immunoglobulin. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable Domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0018] The epitope-binding domain of such antibodies may comprise either complete Variable Domains fused onto Constant Domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the Variable Domains. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an

immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A.F. *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable regions can be “reshaped” or “humanized” by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” Nature 332:323-327; Verhoeyen, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) “*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*,” Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) “*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*,” Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) “*Reshaping A Therapeutic CD4 Antibody*,” Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) “*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*,” Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) “*Humanized Antibodies For Antiviral Therapy*,” Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) “*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*,” Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) “*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*,” J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies).

In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

[0019] A number of “humanized” antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Winter *et al.* (1991) “*Man-made Antibodies*,” *Nature* 349:293-299; Lobuglio *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 86:4220-4224 (1989), Shaw *et al.* (1987) “*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*,” *J. Immunol.* 138:4534-4538, and Brown *et al.* (1987) “*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*,” *Cancer Res.* 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain (see, for example, Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” *Nature* 332:323-327; Verhoeyen, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” *Science* 239:1534-1536; and Jones *et al.* (1986) “*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*,” *Nature* 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These “humanized” molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) “*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*,” *Nucl. Acids Res.* 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

B. Bi-Specific Antibodies, Multi-Specific Diabodies and DART™ Diabodies

[0020] Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are “mono-specific”), although they may be able to bind multiple copies of that species (*i.e.*, they may exhibit bi-valency or multi-valency). A wide variety of recombinant bi-specific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse the antibody core (IgA, IgD, IgE, IgG or IgM) to a further binding protein (*e.g.*, scFv, VL VH, *etc.*) to, or within, the antibody core, or to fuse multiple antibody portions or to fuse (*e.g.* two Fab fragments or scFv) to a Heterodimerization-Promoting Domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). Typically, such approaches involve compromises and trade-offs. For example, PCT Publications Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose that the use of linkers may cause problems in therapeutic settings, and teaches a tri-specific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. Thus, the molecules disclosed in these documents trade binding specificity for the ability to bind additional antigen species. PCT Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. The document notes that the CH2 Domain likely plays only a minimal role in mediating effector function. PCT Publications Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Domains have been replaced with additional VL and VH Domains, so as to form tri-valent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv domains. PCT Publications No. WO 2013/006544 discloses multi-valent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. Thus, the molecules disclosed in these

documents trade all or some of the capability of mediating effector function for the ability to bind additional antigen species. PCT Publications Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional Binding Domains or functional groups to an antibody or an antibody portion (e.g., adding a diabody to the antibody's Light Chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

[0021] The art has additionally noted the capability to produce **diabodies** that differ from such natural antibodies in being capable of binding two or more different epitope species (i.e., exhibiting bi-specificity or multispecificity in addition to bi-valency or multi-valency) (see, e.g., Holliger *et al.* (1993) “*Diabodies*: *Small Bivalent And Bispecific Antibody Fragments*,” Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*,” Protein Eng Des Sel. 17(1):21-27; Wu, A. *et al.* (2001) “*Multimerization Of A Chimeric Anti-CD20 Single-chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*,” Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*,” Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) “*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*,” Cancer Res. 69(12):4941-4944).

[0022] The design of a diabody is based on the structure of single-chain Variable Domain fragments (**scFv**). Such molecules are made by linking light and/ or Heavy Chain Variable Domains to one another via a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes an example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0023] United States Patent No. 7,585,952 and United States Patent Publication No. 2010-0173978 concern scFv molecules that are immunospecific for ErbB2. Bi-specific T cell engagers ("**BiTEs**"), a type of scFv molecule has been described (WO 05/061547; Baeuerle, P *et al.* (2008) "*BiTE: A New Class Of Antibodies That Recruit T Cells*," Drugs of the Future 33: 137-147; Bargou, *et al.* 2008) "*Tumor Regression in Cancer Patients by Very Low Doses of a T Cell-Engaging Antibody*," Science 321: 974-977). Such molecules are composed of a single polypeptide chain molecule having two Antigen-Binding Domains, one of which immunospecifically binds to a CD3 epitope and the second of which immunospecifically binds to an antigen present on the surface of a target cell.

[0024] The provision of non-mono-specific diabodies provides a significant advantage: the capacity to co-ligate and co-localize cells that express different epitopes. Bivalent diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bi-valency allows for great flexibility in the design and

engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) “*Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris*,” Protein Eng. 10:1221). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T cells to tumor cells (Staerz *et al.* (1985) “*Hybrid Antibodies Can Target Sites For Attack By T Cells*,” Nature 314:628-631, and Holliger *et al.* (1996) “*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*,” Protein Eng. 9:299-305).

[0025] Diabody epitope-binding domains may be directed to a surface determinant of any immune effector cell such as CD3, CD16, CD32, CD64, *etc.*, which are expressed on T lymphocytes, Natural Killer (NK) cells or other mononuclear cells. In many studies, diabody binding to effector cell determinants, *e.g.*, Fc γ receptors (Fc γ R), was also found to activate the effector cell (Holliger *et al.* (1996) “*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*,” Protein Eng. 9:299-305; Holliger *et al.* (1999) “*Carcinoembryonic Antigen (CEA)-Specific T-cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-CEA Bispecific Diabodies And B7 x Anti-CEA Bispecific Fusion Proteins*,” Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen bound antibody to an effector cell via Fc-Fc γ R interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.*, as assayed in any effector function assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cells but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) “*Bispecific Antibody Conjugates In Therapeutics*,” Adv. Drug. Deliv. Rev. 55:171-197).

[0026] For example, United States Patent No. 6,171,586, concerns the production of bi-specific antibodies by proteolytically cleaving two antibodies to obtain their F(ab')₂ fragments, reducing such fragments under conditions for preventing intermolecular disulfide bond formation, and then mixing the fragments to generate the bi-specific antibody). United States Patents No. 6,551,592; 6,994,853 and 8,277,806 and PCT Publications Nos. WO 2012/156430, WO 2002/020039, WO 2000/018806 and WO 1998/003670 concern the production of tri-specific antibodies capable of simultaneously binding to T cells and other antigens on a tumor cell, and, via the Fc portion of the bi-specific antibody, to the Fc receptor of cells possessing such a receptor. PCT Publications Nos. WO 2000/018806, WO 1998/003670 and WO 2006/072152 concern the production of tri-specific antibodies capable of simultaneously binding to T cells and other antigens. United States Patent Publication No. 2008-0057054 discloses bi-specific conjugates specific for a binding element against amyloid beta oligomers and a binding element against transmembrane protein telencephalin. United States Patent Publication No. 2010-0291112 concerns bi-specific and tri-specific single-chain Fv molecules that specifically bind to a one (or two) tumor antigen(s) and an effector cell antigen (such as CD3, CD16 CD32, CD64, *etc.*).

[0027] PCT Publication Nos. WO 1999/042597 and WO 1998/006749 disclose antibody derivatives that comprise human Major Histocompatibility Complex binding domains, with or without bound MHC binding peptides. PCT Publication No. WO 02/072141 concerns multi-specific binding molecules whose on-rates (rates at which they bind to target molecules) and off-rates (rates at which they release target molecules) differ so as to preferentially bind to one target compared to their binding to the other such target molecule. Tri-specific molecules, for example molecules having a monovalent first portion which is an Anti-CD3 or anti-CD28 antibody, and a second portion comprising a divalent immune function exerting moiety which immunospecifically binds to one or more target ligands on a target diseased cell or immune cell.

[0028] United States Patent No. 7,695,936 and Patent Publication 2007/ 0196363 concern bi-specific antibodies that are formed from the heavy chains of two

antibodies, one of which possess a protuberance engineered into its heavy chain and the second of which possess a complementary cavity engineered into its heavy chain. The presence of such complementary “knobs” and “holes” is taught to preferentially form bi-specific hetero-antibodies (having one heavy chain of each such antibody) relative to mono-specific homo-antibodies that contain two heavy chains of the same antibody. Various bi-specific hetero-antibodies are proposed, including those that are immunospecific for CD3 and a tumor cell antigen. Various tri-specific hetero-antibodies are also proposed, including some that are immunospecific for CD3, CD8 and CD37 (a transmembrane protein expressed predominantly on B cells that is involved the regulation of T cell proliferation (Robak, T. *et al.* (2014) “Anti-CD37 Antibodies For Chronic Lymphocytic Leukemia,” *Expert Opin. Biol. Ther.* 14(5):651-661), however, no mechanism for their production and no disclosure of their structure is provided.

[0029] PCT Publication WO2012-162561 concerns bi-specific, tetravalent binding molecules that comprise two polypeptides, each of which comprises two diabody structures, separated by an intervening CH2-CH3 Domain. The document also concerns tetravalent binding molecules composed of four polypeptide chains in which two of the polypeptide chains contain variable light and variable heavy Domains for two antigens, and in which the other two polypeptide chains contain the complementary variable heavy and variable light Domains for the antigens and a terminal CH2-CH3 Domain. The bi-specific, tetravalent binding molecules form through the association of their respective CH2-CH3 Domains. In the four polypeptide chain construct, the “light” chains are not covalently bound to the heavy chains, thus leading to instability (see, Lu, D. *et al.* (2005) “A Fully Human Recombinant IgG-like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,” *J. Biol. Chem.* 280(20):19665-19672). The document discloses a third construct in which the chains are altered to provide such covalent bonding, but at the cost of eliminating their bi-specificity (*i.e.*, the molecules are mono-specific). Molecules having specificity for CD2, CD3, CD4, CD8, CD161, a chemokine receptor, CD95, CCR5, *etc.* are disclosed. A bi-specific molecule capable of binding to both CD3 and CD8 is not disclosed.

[0030] However, the above advantages come at salient cost. The formation of such *non-mono-specific* diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to mono-specific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-mono-specific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*,” Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*,” Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” J. Biol. Chem. 280(20):19665-19672).

[0031] However, the art has recognized that bi-specific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” J. Biol. Chem. 280(20):19665-19672).

[0032] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-mono-specific diabodies, termed **DARTs™** (see, e.g., United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Moore, P.A. *et al.* (2011) “*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma,*” *Blood* 117(17):4542-4551; Veri, M.C. *et al.* (2010) “*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold,*” *Arthritis Rheum.* 62(7):1933-1943; Johnson, S. *et al.* (2010) “*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion,*” *J. Mol. Biol.* 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond two polypeptide chains. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0033] There are many DART™ embodiments. Each of the two polypeptides of the simplest DART™ embodiment comprises three Domains (**Figure 1**). The first polypeptide comprises: (i) a first domain that comprises a binding region of a Light Chain Variable Domain of the a first immunoglobulin (VL1), (ii) a second domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), and (iii) a third domain that contains a cysteine residue (or a Cysteine-Containing Domain) and a Heterodimerization-Promoting Domain that serves to promote heterodimerization with the second polypeptide chain. The cysteine residue (or a Cysteine-Containing Domain) of the third domain serves to promote the covalent bonding of the first polypeptide chain to the second polypeptide chain of the diabody. The second polypeptide contains: (i) a complementary first domain (a VL2-containing Domain), (ii) a complementary second domain (a VH1-

containing Domain) and (iii) a third domain that contains a cysteine residue (or a Cysteine-Containing Domain) and, optionally, a complementary Heterodimerization-Promoting Domain that complexes with the Heterodimerization-Promoting Domain of the first polypeptide chain in order to promote heterodimerization with the first polypeptide chain. The cysteine residue (or a Cysteine-Containing Domain) of the third domain of the second polypeptide chain serves to promote the covalent bonding of the second polypeptide chain to the first polypeptide chain of the diabody. Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. They are able to promote re-directed T cell mediated killing of cells expressing target antigens.

[0034] In one embodiment, the third domains of the first and second polypeptides each contain a cysteine residue, which serves to bind the polypeptides together via a disulfide bond. The third domain of one or both of the polypeptides may additionally possess the sequence of a CH2-CH3 Domain, such that complexing of the diabody polypeptides forms an Fc Domain that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, Natural Killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells) (**Figures 2A-2B**).

[0035] Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). These Fc-bearing DARTs may comprise three polypeptide chains (*e.g.*, **Figure 2B**). The first polypeptide chain of such a diabody contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a domain containing a cysteine residue (or a Cysteine-Containing Domain) and a Heterodimerization-Promoting Domain, and (iv) a cysteine residue (or a Cysteine-Containing Domain and a CH2-CH3 Domain). The second polypeptide chain of such DARTTM contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that contains a cysteine residue (or a Cysteine-Containing Domain) and a Heterodimerization-Promoting Domain that promotes heterodimerization with the first polypeptide chain. The cysteine residue (or a Cysteine-Containing Domain) of

the third domain of the second polypeptide chain serves to promote the covalent bonding of the second polypeptide chain to the first polypeptide chain of the diabody. The third polypeptide of such DART™ comprises a cysteine residue (or a Cysteine-Containing Domain) and a CH2-CH3 Domain. Thus, the first and second polypeptide chains of such DART™ associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective third domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain that is stabilized via a disulfide bond. Such diabodies have enhanced potency. Such Fc-bearing **DARTs™** may have either of two orientations (**Table 1**):

Table 1		
First Orientation	3 rd Chain	NH ₂ -CH2-CH3-COOH
	1 st Chain	NH ₂ -VL1-VH2-Cys-Heterodimer-Promoting Domain-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL2-VH1-Cys-Heterodimer-Promoting Domain-COOH
Second Orientation	3 rd Chain	NH ₂ -CH2-CH3-COOH
	1 st Chain	NH ₂ -CH2-CH3-VL1-VH2-Cys-Heterodimer-Promoting Domain-COOH
	2 nd Chain	NH ₂ -VL2-VH1-Cys-Heterodimer-Promoting Domain-COOH

[0036] Even more complex DART™ diabodies, termed **Ig-DART™** (**Figures 3A-3B**) and **Fc-DART™** (**Figure 3C**) have been described (WO 2012/018687). Fc-DARTs™ have four polypeptide chains. The first and third polypeptide chains of such a diabody contain three Domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide of the Fc-DART™ contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the Fc-DART's™ first polypeptide chain. The third and fourth, and the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either mono-specific, bi-specific or tetra-specific. Such more complex DART™ molecules also possess Cysteine-Containing Domains which function to form a covalently bonded complex. Fc-DART™ diabodies contain CH1 and CL Domains.

[0037] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required including, but not limited to, tetravalent tandem antibodies, also referred to as “**TandAbs**” (see, *e.g.* United States Patent Publications Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publications Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical chains each possessing a VH1, VL2, VH2, and VL2 Domain.

[0038] However, despite all prior advances, a need remains for compositions that could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to this and other goals.

Summary:

[0039] The present disclosure relates to Tri-Specific Binding Molecules, which are multi-chain polypeptide molecules that possess three Binding Domains and are thus capable of mediating coordinated binding to three epitopes. The Tri-Specific Binding Molecule is preferably characterized in possessing binding domains that permit it to immunospecifically bind to: (1) an epitope of a first Cancer Antigen, (2) an epitope of a second Cancer Antigen, and (3) an epitope of a molecule that is expressed on the surface of an immune system effector cell, and are thus capable of localizing an immune system effector cell to a cell that expresses a Cancer Antigen, so as to thereby facilitate the killing of such cancer cell.

[0040] In detail, the disclosure provides a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, said Epitopes being Epitope I, Epitope II, and Epitope III, wherein two of three epitopes are epitopes of Cancer Antigen(s), and the third of said epitopes is an epitope of an Effector Cell Antigen.

[0041] The disclosure particularly concerns the embodiment of such Tri-Specific Binding Molecule wherein the molecule comprises four different polypeptide chains covalently complexed together and comprises:

- (I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that

is capable of immunospecifically binding to an Epitope II present on a second antigen, wherein the Antigen-Binding Domain I and the Antigen-Binding Domain II are both Diabody-Type Binding Domains;

- (II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and
- (III) an Fc Domain that is formed by the complexing of two CH2-CH3 Domains to one another;

wherein one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and the third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen, and wherein the Antigen-Binding Domains I, II and III of the Binding Molecules mediate coordinated binding of an immune system effector cell expressing the Effector Cell Antigen and a cancer cell expressing the first and second Cancer Antigens.

[0041a] In one aspect, there is provided a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:

- (I) four different polypeptide chains covalently complexed together;
- (II) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;
- (III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and
- (IV) a Fc Domain, wherein:
 - (A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) a fourth polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{III} Domain)—(Cysteine-Containing Domain);

(F) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III}

Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III: and

(G) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

[0041b] In another aspect, there is provided a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:

(I) three different polypeptide chains covalently complexed together;

(II) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(IV) a Fc Domain; wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain); or

(Cysteine-Containing Domain 1)—(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain 2)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III; and

(F) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III}

Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

[0042] The disclosure particularly concerns the embodiment of such Tri-Specific Binding Molecules wherein the Fc Domain is capable of binding to an Fc Receptor arrayed on the surface of a cell.

[0043] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Effector Cell Antigen is arrayed on the surface of an effector cell and wherein the Cancer Antigens are arrayed on the surface of a cancer cell, and wherein the immunospecific binding is sufficient to co-localize the Effector Cell Antigen, and the Cancer Antigens, thereby facilitating the activation of the effector cell against the cancer cell.

[0044] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Effector Cell Antigen is selected from the group consisting of: CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, the B cell Receptor (BCR), the T cell Receptor (TCR), and the NKG2D Receptor.

[0045] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the first and second Cancer Antigens are independently selected from the group consisting of: colon cancer antigen 19.9; a gastric cancer

mucin; antigen 4.2; glycoprotein A33 (gpA33); ADAM-9; gastric cancer antigen AH6; ALCAM; malignant human lymphocyte antigen APO-1; cancer antigen B1; B7-H3; beta-catenin; blood group ALe^b/Le^y; Burkitt's lymphoma antigen-38.13, colonic adenocarcinoma antigen C14; ovarian carcinoma antigen CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD30 ; CD33; CD36; CD45; CD46; CD52; CD79a/CD79b; CD103; CD317; CDK4; carcinoembryonic antigen (CEA); CEACAM5; CEACAM6; CO17-1A; CO-43 (blood group Le^b); CO-514 (blood group Le^a); CTA-1; CTLA4; Cytokeratin 8; antigen D1.1; antigen D₁₅₆₋₂₂; DR5; E₁ series (blood group B); EGFR (Epidermal Growth Factor Receptor); Ephrin receptor A2 (EphA2); ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; lung adenocarcinoma antigen F3; antigen FC10.2; G49, ganglioside GD2; ganglioside GD3; ganglioside GM2; ganglioside GM3; G_{D2}; G_{D3}; GICA 19-9; G_{M2}; gp100; human leukemia T cell antigen Gp37; melanoma antigen gp75; gpA33; HER2 antigen (p185^{HER2}); human milk fat globule antigen (HMFG); human papillomavirus-E6/human papillomavirus-E7; high molecular weight melanoma antigen (HMW-MAA); I antigen (differentiation antigen) I(Ma); Integrin Alpha-V-Beta-6 Integrinβ6 (ITGB6); Interleukin-13; Receptor α2 (IL13Rα2); JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; human lung carcinoma antigens L6 and L20; LEA; LUCA-2; M1:22:25:8; M18; M39; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; Myl; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; melanoma-associated antigen p97; polymorphic epithelial mucin (PEM); polymorphic epithelial mucin antigen (PEMA); PIPA; prostate-specific antigen (PSA); prostate-specific membrane antigen (PSMA); prostatic acid phosphate; R₂₄; ROR1; sphingolipids; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; T_{5A7}; TAG-72; TL5 (blood group A); TNF-α receptor; TNF-β receptor; TNF-γ receptor; TRA-1-85 (blood group H); Transferrin Receptor; tumor-specific transplantation antigen (TSTA), oncofetal antigen-alpha-fetoprotein (AFP); VEGF; VEGFR, VEP8; VEP9; VIM-D5; and Y hapten, Le^y.

[0046] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the first and second Cancer Antigens are selected from the group consisting of: CD2, CD317, CEACAM5, CEACAM6, DR5, EphA2, gpA33, Her2, B7-H3; EGF, EGFR, VEGF and VEGFR.

[0047] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Non-Diabody-Type Binding Domain III comprises the Fab-Type Binding Domain (VL_{III}/VH_{III}) that is capable of immunospecifically binding to an Epitope III, wherein the molecule comprises:

(A) a first polypeptide chain that comprises in the N-terminus to C-terminus direction:

- (1) a light chain variable Domain of an immunoglobulin capable of binding to a first of the three epitopes (VL_I);
- (2) a heavy chain variable Domain of an immunoglobulin capable of binding to a second of the three epitopes (VH_{II});
- (3) a Heterodimer-Promoting Domain; and
- (4) CH2 and CH3 Domains of an IgG;

(B) a second polypeptide chain that comprises, in the N-terminus to C-terminus direction:

- (1) a light chain variable Domain of an immunoglobulin capable of binding to the second of the three epitopes (VL_{II});
- (2) a heavy chain variable Domain of an immunoglobulin capable of binding to the first of the three epitopes (VH_I); and
- (3) a complementary Heterodimer-Promoting Domain;

(C) a third polypeptide chain that comprises, in the N-terminus to C-terminus direction:

- (1) a heavy chain variable Domain of an immunoglobulin capable of binding to a third of the three epitopes (VH_{III}); and
- (2) a CH1 Domain, a Hinge Domain, and a CH2-CH3 Domain of an IgG;

and

(D) a fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:

- (1) a light chain variable Domain of an immunoglobulin capable of binding to the third of the three epitopes (VL_{III}); and
- (2) a light chain constant Domain (CL);

wherein:

- (i) the VL_I and VH_I Domains associate to form a Domain capable of binding the first epitope;
- (ii) the VL_{II} and VH_{II} Domains associate to form a Domain capable of binding the second epitope;
- (iii) the VL_{III} and VH_{III} Domains associate to form a Domain capable of binding the third epitope;
- (iv) the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form an Fc Domain;
- (v) the first and second polypeptide chains are covalently bonded to one another;
- (vi) the first and third polypeptide chains are covalently bonded to one another; and
- (vii) the third and fourth polypeptide chains are covalently bonded to one another.

[0048] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

- (A) the Heterodimer-Promoting Domain is an E-coil and the complementary Heterodimer-Promoting Domain is a K-coil; or
- (B) the Heterodimer-Promoting Domain is a K-coil and the complementary Heterodimer-Promoting Domain is an E-coil.

[0049] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

- (A) the CH2-CH3 Domains of the first and third polypeptide chains each have the sequence of **SEQ ID NO:1**, such that the Fc Domain formed from their association exhibits normal FcγR-mediated effector function; or
- (B) the CH2-CH3 Domain of the first and third polypeptide chains comprise at least one amino acid substitution, relative to the sequence of **SEQ ID NO:1**, such that the Fc Domain formed from their association exhibits altered FcγR-mediated effector function.

[0050] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the at least one amino acid substitution comprises at least one amino acid substitution selected from the group consisting of: L235V, F243L, R292P, Y300L, V305I, and P396L, wherein the numbering is that of the EU index as in Kabat.

[0051] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the at least one amino acid substitution comprises:

- (A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;
 - (B) at least two substitutions selected from the group consisting of:
 - (1) F243L and P396L;
 - (2) F243L and R292P; and
 - (3) R292P and V305I;
 - (C) at least three substitutions selected from the group consisting of:
 - (1) F243L, R292P and Y300L;
 - (2) F243L, R292P and V305I;
 - (3) F243L, R292P and P396L; and
 - (4) R292P, V305I and P396L;
 - (D) at least four substitutions selected from the group consisting of:
 - (1) F243L, R292P, Y300L and P396L; and
 - (2) F243L, R292P, V305I and P396L;
- or
- (E) at least the five substitutions selected from the group consisting of:
 - (1) F243L, R292P, Y300L, V305I and P396L; and
 - (2) L235V, F243L, R292P, Y300L and P396L.

[0052] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the CH2-CH3 Domain of the first and third polypeptide chains differ from one another and have an amino acid sequence selected from the group consisting of **SEQ ID NO:52** and **SEQ ID NO:53**.

[0053] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

- (A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the second Cancer Antigen and an epitope of the Effector Cell Antigen;
 - (B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the second Cancer Antigen;
 - (C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of second Cancer Antigen, an epitope of the first Cancer Antigen, and an epitope of the Effector Cell Antigen;
 - (D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the second Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the first Cancer Antigen;
 - (E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of the first Cancer Antigen, and an epitope of the second Cancer Antigen;
- and
- (F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of second Cancer Antigen, and an epitope of the first Cancer Antigen.

[0054] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

- (A) the epitope of an Effector Cell Antigen is a CD2 epitope recognized by antibody Lo-CD2a;
- (B) the epitope of an Effector Cell Antigen is a CD3 epitope recognized by antibody OKT3, M291, YTH12.5, Anti-CD3 mAb 1 or Anti-CD3 mAb 2;
- (C) the epitope of an Effector Cell Antigen is a CD16 epitope recognized by antibody 3G8 or A9;
- (D) the epitope of an Effector Cell Antigen is a CD19 epitope recognized by antibody MD1342, MEDI-551, blinatumomab or HD37;

- (E) the epitope of an Effector Cell Antigen is a CD20 epitope recognized by antibody rituximab, ibritumomab, ofatumumab, and tositumomab;
 - (F) the epitope of an Effector Cell Antigen is a CD22 epitope recognized by antibody epratuzumab;
 - (G) the epitope of an Effector Cell Antigen is a CD32B epitope recognized by antibody CD32B mAb 1;
 - (H) the epitope of an Effector Cell Antigen is a CD64 epitope recognized by antibody CD64 mAb 1;
 - (I) the epitope of an Effector Cell Antigen is a BCR/CD79 epitope recognized by antibody CD79 mAb 1;
 - (J) the epitope of an Effector Cell Antigen is a TCR epitope recognized by antibody BMA 031;
- or
- (K) the epitope of an Effector Cell Antigen is a NKG2D Receptor epitope recognized by antibody KYK-2.0.

[0054a] In one aspect, there is provided a nucleic acid composition comprising polynucleotides that encode polypeptide chains of any of the above-described Tri-Specific Binding Molecules.

[0055] In another aspect, there is provided a pharmaceutical composition comprising any of the above-described Tri-Specific Binding Molecules, and a pharmaceutically acceptable carrier, excipient or diluent.

[0056] In another aspect, there is provided any of the above-described Tri-Specific Binding Molecules, or a composition as above-described, for use as a medicament. In yet another aspect, there is provided use of any of the above-described Tri-Specific Binding Molecules or a composition as above-described in the manufacture of a medicament to treat a cancer.

[0057] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma,

a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0058] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules wherein the cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer or a rectal cancer.

[0059] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules the cancer is acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantle cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

Brief Description of the Drawings:

[0060] **Figures 1A-1B** show diagrammatic representation of the Domains of DART™ diabodies. **Figure 1A** shows a diagrammatic representation of the Domains of a basic DART™ diabody. **Figure 1B** provides a schematic of a covalently bonded diabody composed of two polypeptide chains, each having a Heterodimer-Promoting Domain VL and VH domains that recognize the same epitope are shown using the same shading.

[0061] **Figures 2A-2B** provide a schematic of covalently bonded diabodies composed of two polypeptide chains, each having a CH2 and CH3 Domain (**Figure 2A**) or in which only one has a CH2 and CH3 Domain (**Figure 2B**), such that the associated chains form an Fc Domain that comprises all or part of a naturally occurring Fc Domain. VL and VH domains that recognize the same epitope are shown using the same shading.

[0062] **Figures 3A-3C** provide schematics showing tetravalent diabodies composed of two pairs of polypeptide chains. The pairs are different, thus resulting in a bi-specific molecule that is bivalent with respect to each of two epitopes, in which one is an epitope of DR5 and the other is an epitope of a molecule present on the surface of an effector cell. One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form an Fc Domain that comprises all or part of a naturally occurring Fc Domain. VL and VH domains that recognize the same epitope are shown using the same shading. Only one pair of epitopes (shown with the same shading) is capable of binding to DR5. **Figure 3A** shows an Ig diabody. **Figure 3B** shows an Ig diabody, which contains E-coil and K-coil heterodimer-promoting domains. **Figure 3C**, shows an Fc-DART™ diabody that contains antibody CH1 and CL domains. The notation “VL1” and “VH1” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the “first” epitope. Similarly, the notation “VL2” and “VH2” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the “second” epitope.

[0063] **Figures 4A-4G** provide a diagrammatic representation of the Domains of preferred Tri-Specific Binding Molecules of the present invention. The Figures illustrate schematically the order and orientation of the Domains of embodiments of the preferred Tri-Specific Binding Molecules of the present invention. **Figures 4A, 4B and 4G** illustrate embodiments in which the Tri-Specific Binding Molecule is composed of four polypeptide chains. **Figures 4C, 4D, 4E and 4F** illustrate embodiments in which the binding molecule is composed of three polypeptide chains. The molecule may possess Hinge and/or CL domains (**Figures 4A, 4B, 4C, 4E**) or may contain an alternative linker peptide (**Figure 4D, 4F, 4G**).

[0064] **Figures 5A-5E** provide a diagrammatic representation of the Domains of an alternative embodiment of the Tri-Specific Binding Molecules of the present invention, in which the Effector Cell-Binding Domain is composed of an Effector Cell Receptor-Type Binding Domain rather than a Diabody-Type Binding Domain or a Fab-Type Binding Domain. **Figures 5A and 5B** illustrate embodiments in which the Tri-Specific Binding Molecule is composed of four polypeptide chains. **Figure 5C and Figure 5E** illustrate an embodiment in which the binding molecule is composed of three polypeptide chains. **Figure 5D** illustrates an embodiment in which the binding molecule is composed of five polypeptide chains. The molecule may possess Hinge and/or CL domains or may contain alternative linker peptides.

[0065] **Figure 6** shows the ability of anti-human DR5 monoclonal antibodies DR5 mAb 1 and DR5 mAb 2 to bind to human DR5 and to the DR5 of cynomolgus monkey.

[0066] **Figure 7, Panels A-H**, show the kinetics of binding of DR5 mAb 1 (**Panels A and E**), DR5 mAb 2 (**Panels B and F**), DR5 mAb 3 (**Panels C and G**) and DR5 mAb 4 (**Panels D and H**) for human DR 5 (**Panels A-D**) and for cynomolgus monkey DR5 (**Panels E-H**).

[0067] **Figure 8** shows the unexpected superiority of DR5 mAb 1 and DR5 mAb 2. Superiority was assessed by comparing the ability of DR5 x CD3 diabodies having the VL and VH Domains of DR5 mAb 1, DR5 mAb 2, DR5 mAb 3, or DR5 mAb 4,

to mediate the cytotoxicity of A549 adenocarcinomic human alveolar basal epithelial tumor cells.

[0068] **Figures 9A-9C** demonstrate the synergistic enhancement in target cell binding that is attained when both of the two Cancer Antigen-Binding Domains of a Tri-Specific Binding Molecule of the present invention are able to bind to a target cell. **Figure 9A** shows the binding obtained when trispecific molecules: EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1; EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1; and gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 are incubated in the presence of EphA2-expressing CHO cells. **Figure 9B** shows the binding obtained when such trispecific molecules are incubated in the presence of DR5-expressing CHO cells. **Figure 9C** shows the binding obtained when such trispecific molecules are incubated in the presence of DU145 human prostate cells that express EphA2 and DR5, but not gpA33.

[0069] **Figures 10A-10C** demonstrate the synergistic enhancement in target cell cytotoxicity that is attained when both of the two Cancer Antigen-Binding Domains of a Tri-Specific Binding Molecule of the present invention are able to bind to a target cell. **Figure 10A** shows the percent cytotoxicity obtained by incubating trispecific molecules: EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1; EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1; and gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 are incubated in the presence of EphA2-expressing CHO cells and cytotoxic lymphocytes. **Figure 10B** shows the percent cytotoxicity obtained when such trispecific molecules are incubated in the presence of DR5-expressing CHO cells and cytotoxic lymphocytes. **Figure 10C** shows the cytotoxicity obtained when such trispecific molecules are incubated in the presence of DU145 human prostate cells and cytotoxic lymphocytes. DU145 cells express EphA2 and DR5, but not gpA33. Cytotoxicity was measured by the increase in luminescence caused by the release of luciferase upon cell lysis.

Detailed Description of the Invention:

[0070] The present invention relates to Tri-Specific Binding Molecules, which are multi-chain polypeptide molecules that possess three Binding Domains and are thus capable of mediating coordinated binding to three epitopes. The Tri-Specific Binding

Molecule is preferably characterized in possessing binding domains that permit it to immunospecifically bind to: (1) an epitope of a first Cancer Antigen, (2) an epitope of a second Cancer Antigen, and (3) an epitope of a molecule that is expressed on the surface of an immune system effector cell, and are thus capable of localizing an immune system effector cell to a cell that expresses a Cancer Antigen, so as to thereby facilitate the killing of such cancer cell.

[0071] The Tri-Specific Binding Molecules of the present invention may include Epitope-Binding Domains of humanized, chimeric or caninized derivatives of the above-discussed antibodies, for example, DR5 mAb 1 or DR5 mAb 2.

I. General Techniques and General Definitions

[0072] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, MOLECULAR CLONING: A LABORATORY MANUAL, Third Edition (Sambrook *et al.* Eds., 2001) Cold Spring Harbor Press, Cold Spring Harbor, NY; OLIGONUCLEOTIDE SYNTHESIS: METHODS AND APPLICATIONS (Methods in Molecular Biology), Herdewijn, P., Ed., Humana Press, Totowa, NJ; OLIGONUCLEOTIDE SYNTHESIS (Gait, M.J., Ed., 1984); METHODS IN MOLECULAR BIOLOGY, Humana Press, Totowa, NJ; CELL BIOLOGY: A LABORATORY NOTEBOOK (Cellis, J.E., Ed., 1998) Academic Press, New York, NY; ANIMAL CELL CULTURE (Freshney, R.I., Ed., 1987); INTRODUCTION TO CELL AND TISSUE CULTURE (Mather, J.P. and Roberts, P.E., Eds., 1998) Plenum Press, New York, NY; CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Doyle, A. *et al.*, Eds., 1993-8) John Wiley and Sons, Hoboken, NJ; METHODS IN ENZYMOLOGY (Academic Press, Inc.) New York, NY; WEIR'S HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (Herzenberg, L.A. *et al.* Eds. 1997) Wiley-Blackwell Publishers, New York, NY; GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (Miller, J.M. *et al.* Eds., 1987) Cold Spring Harbor Press, Cold Spring Harbor, NY; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Ausubel, F.M. *et al.*, Eds., 1987) Greene Pub. Associates, New York, NY; PCR: THE POLYMERASE CHAIN REACTION, (Mullis, K. *et al.*, Eds., 1994) Birkhäuser, Boston MA; CURRENT PROTOCOLS IN

IMMUNOLOGY (Coligan, J.E. *et al.*, eds., 1991) John Wiley and Sons, Hoboken, NJ; SHORT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley and Sons, 1999) Hoboken, NJ; IMMUNOBIOLOGY 7 (Janeway, C.A. *et al.* 2007) Garland Science, London, UK; Antibodies (P. Finch, 1997) Stride Publications, Devoran, UK; ANTIBODIES: A PRACTICAL APPROACH (D. Catty., ed., 1989) Oxford University Press, USA, New York NY); MONOCLONAL ANTIBODIES: A PRACTICAL APPROACH (Shepherd, P. *et al.* Eds., 2000) Oxford University Press, USA, New York NY; USING ANTIBODIES: A LABORATORY MANUAL (Harlow, E. *et al.* Eds., 1998) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; THE ANTIBODIES (Zanetti, M. *et al.* Eds. 1995) Harwood Academic Publishers, London, UK); and DEVITA, HELLMAN, AND ROSENBERG'S CANCER: PRINCIPLES & PRACTICE OF ONCOLOGY, EIGHTH EDITION, DeVita, V. *et al.* Eds. 2008, Lippincott Williams & Wilkins, Philadelphia, PA.

II. Preferred Tri-Specific Binding Molecules of the Present Invention

A. Binding Capabilities

[0073] The preferred Tri-Specific Binding Molecules of the present invention are able to coordinately and simultaneously bind to three different epitopes. The preferred Tri-Specific Binding Molecules of the present invention comprise:

- (I) a “**Binding Domain I**” that is capable of immunospecifically binding to an “**Epitope I**” present on a first antigen, and a “**Binding Domain II**” that is capable of immunospecifically binding to an “**Epitope II**” present on a second antigen, wherein said **Binding Domain I** and said **Binding Domain II** are both “**Diabody-Type Binding Domains**,”
- (II) a “**Binding Domain III**” that is capable of immunospecifically binding to an “**Epitope III**” present on a third antigen; and
- (III) an **Fc Domain** that is formed by the complexing of two **CH2-CH3 Domains** to one another;

wherein:

- (A) one of Epitope I, Epitope II or Epitope III is an epitope of a first “**Cancer Antigen**” Cancer Antigen;
- (B) a second of Epitope I, Epitope II or Epitope III is an epitope of a second **Cancer Antigen**; and

(C) the third of Epitope I, Epitope II or Epitope III is an epitope of a molecule expressed on the surface of an immune system effector cell ("**Effector Cell Antigen**");

and wherein the Binding Domains I, II and III of the binding molecules mediate coordinated binding of the immune system effector cell and a cell expressing both the first and second Cancer Antigens to thereby co-localize such cells.

[0074] Diabody Epitope-Binding Domains may also be directed to a surface determinant of a B cell, such as CD19, CD20, CD22, CD30, CD37, CD40, and CD74 (Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Cheson, B.D. *et al.* (2008) "*Monoclonal Antibody Therapy For B-Cell Non-Hodgkin's Lymphoma*," N. Engl. J. Med. 359(6):613-626; Castillo, J. *et al.* (2008) "*Newer monoclonal antibodies for hematological malignancies*," Exp. Hematol. 36(7):755-768. In many studies, diabody binding to effector cell determinants, *e.g.*, Fc γ receptors (Fc γ R), was also found to activate the effector cell (Holliger *et al.* (1996) "*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bi-specific Diabody*," Protein Eng. 9:299-305; Holliger *et al.* (1999) "*Carcinoembryonic Antigen (CEA)-Specific T-Cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-CEA Bi-specific Diabodies And B7 x Anti-CEA Bi-specific Fusion Proteins*," Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen bound antibody to an effector cell via Fc-Fc γ R interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.*, as assayed in any effector function assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cells but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) "*Bi-specific Antibody Conjugates In Therapeutics*," Adv. Drug. Deliv. Rev. 55:171-197).

[0075] Although such Tri-Specific Binding Molecules are particularly preferred, the invention additionally specifically contemplates Tri-Specific Binding Molecules

that comprise any combination of Binding Domains sufficient to produce a molecule having three binding specificities, of which two are binding specificities directed against Cancer Antigens, and one is a binding specificity directed against an Effector Cell Antigen. Thus, for example, the invention contemplates: a Tri-Specific Binding Molecule that comprises three Fab-Type Binding Domains, a Tri-Specific Binding Molecule that comprises one bivalent, bi-specific antibody domain (formed for example, by complexing two different light chains and two different heavy chains) and one Fab-Type Binding Domain, a Tri-Specific Binding Molecule that comprises two bivalent, bi-specific antibody domains (formed for example, by complexing four different light chains and two different heavy chains), but in which one of antibody domains has been rendered inactive, *etc.*

[0076] The terms “**polypeptide**,” “**polypeptide chain**,” and “**peptide**” are used interchangeably herein to refer to polymers of amino acids of any length, but especially lengths greater than 3, 5, 10, 15, 20 or 25 amino acid residues, in which two, and more preferably all, amino acid residues are joined via an amide (peptide) bond (-NH-C(O)-). The polymer may however be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, *etc.*), as well as other modifications known in the art. The polypeptides of this invention can occur as single-chains or as complexed chains.

[0077] A “**Diabody-Type Binding Domain**” is the Epitope-Binding Domain of a diabody, and especially, a DART® diabody. The terms “**diabody**” and “**DART® diabody**” has been discussed above, and refers to a molecule that comprises at least two polypeptide chains that preferably complex with one another through a covalent interaction to form at least two epitope binding sites, which may recognize the same or different epitopes. Two of the polypeptide chains of a diabody or DART® diabody

each comprise immunoglobulin Light Chain Variable Region and an immunoglobulin Heavy Chain Variable Region, but these regions do not interact to form an epitope binding site (*i.e.*, they are not mutually “**complementary**”). Rather, the immunoglobulin Heavy Chain Variable Region of one (*e.g.*, the first) of the diabody, or DART® diabody, chains interacts with the immunoglobulin Light Chain Variable Region of a different (*e.g.*, the second) diabody or, DART® diabody, polypeptide chain to form an epitope binding site. Similarly, the immunoglobulin Light Chain Variable Region of one (*e.g.*, the first) of the diabody, or DART® diabody, polypeptide chains interacts with the immunoglobulin Heavy Chain Variable Region of a different (*e.g.*, the second) diabody, or DART® diabody, polypeptide chain to form an epitope binding site. DART® diabody molecules are disclosed in United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; WO 2006/113665, WO 2008/157379 and Moore, P.A. *et al.* (2011) “*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*,” Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) “*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcγ Receptor IIb (CD32B) Inhibitory Function With A Novel Bi-specific Antibody Scaffold*,” Arthritis Rheum. 62(7):1933-1943; and Johnson, S. *et al.* (2010) “*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*,” J. Mol. Biol. 399(3):436-449.

[0078] **Binding Domain III** is preferably a “**Non-Diabody-Type**” **Binding Domain**, which is intended to denote that Binding Domain III does not have the structure of a Diabody-Type Binding Domain. Preferably, Binding Domain III is a Non-Diabody-Type Binding Domain that is a **Fab-Type Binding Domain** or an **Effector Cell Receptor-Type Binding Domain**. Thus, in one embodiment, exemplified in **Figures 4A-4G**, the Binding Domain III is a Fab-Type Binding Domain. **Figures 5A-5E** exemplify the embodiment in which Binding Domain III is an Effector Cell Receptor-Type Binding Domain. As used herein, the term an “**Fab-Type Binding Domain**” refers to an epitope Binding Domain that is formed by the interaction of the VL Domain of an immunoglobulin light chain and a complementing

VH Domain of an immunoglobulin heavy chain. Fab-Type Binding Domains differ from Diabody-Type Binding Domain in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single epitope Binding Domain, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two epitope Binding Domains. Thus, as used herein Fab-Type Binding Domains are distinct from Diabody-Type Binding Domain. When a binding domain is a Fab-Type Binding Domain or a Diabody-Type Binding Domain, it will be composed of a VL Domain and a VH Domain, which may be located on the same or on different polypeptide chains. The selection of such VL and VH domains is coordinated, such that the domains form an epitope binding domain. As used herein, the term “**Effector Cell Receptor-Type Binding Domain**” refers to an epitope binding domain that is formed by the interaction of a variable domain of a T Cell Receptor alpha chain and a variable domain of a T Cell Receptor beta chain. Such receptors recognize peptides displayed in the context of MHC and are thus capable of recognizing intracellular epitopes.

[0079] The Tri-Specific Binding Molecules of the present invention are thus distinguished from tetravalent binding molecules, such as those produced from the dimerization of a bivalent antibody, and preferably possess three and not four Binding Domains. As discussed below, the trispecific molecules of the present invention may possess additional binding domains (such as an Albumin-Binding Domain, an FcγR-Binding Domain, *etc.*). Such additional Binding Domains are not intended to be considered or counted as being one of the three Binding Domains of the Tri-Specific Binding Molecules of the present invention.

[0080] As used herein, the terms “**association**” or “**associating**,” with regard to polypeptides (*e.g.*, one diabody polypeptide to another, an immunoglobulin light chain to an immunoglobulin heavy chain, one CH2-CH3 Domain to another CH2-CH3 Domain, *etc.*) is intended to denote a non-covalent combining of the polypeptides. The terms “**complexes**” or “**complexing**” are intended to denote a covalent combining of the polypeptides.

[0081] As used herein, Binding Domains of a Binding Molecule of the invention is said to mediate “**coordinated binding**” if at least two of its Binding Domains and preferably all of its Binding Domains, are capable of concurrently being bound to their respective recognized epitopes or binding ligand. Such binding may be simultaneous. However, one aspect of the present invention relates to modifying the “on” and/or “off” rates with which such Binding Domains bind to their recognized epitopes. As used here, the “**on rate**” of binding is a measure of the affinity with which such Binding Domains recognize and initiate binding to their recognized epitopes. In contrast, the “**off rate**” of binding is a measure of the degree of stability of the Binding Domain:epitope complex. The “on” and/or “off” rates of binding can be modified by altering the amino acid sequence of the CDRs of a Binding Domain. As discussed below, independent of any CDR modifications, the extent of coordinated binding of the molecules of the present invention may be modulated by changing the configuration of the their Binding Domains so that a particular Binding Domain (*i.e.*, a VLx/VHx Domain) is present as Binding Domain III or as an internal or external Diabody-Type Binding Domain relative to Binding Domain III (discussed in detail below).

[0082] The on- and off-rates of the Binding Domains of the Binding Molecules of the present invention can be readily measured by methods well-known in the art, for example by Biacore® analysis (Jason-Moller, L. *et al.* (2006) “*Overview Of Biacore Systems And Their Applications*,” Curr. Protoc. Protein Sci. Chapter 19:Unit 19.13; Swanson, S.J. (2005) “*Characterization Of An Immune Response*,” Dev. Biol. (Basel). 122:95-101; Buijs, J. *et al.* (2005) “*SPR-MS In Functional Proteomics*,” Brief Funct. Genomic Proteomic. 4(1):39-47; Karlsson, R. *et al.* (2004) “*SPR For Molecular Interaction Analysis: A Review Of Emerging Application Areas*,” J. Mol. Recognit. 17(3):151-161; Van Regenmortel, M.H. (2003) “*Improving The Quality Of BIACORE-Based Affinity Measurements*,” Dev. Biol. (Basel) 112:141-151; Malmqvist, M. (1999) “*BIACORE: An Affinity Biosensor System For Characterization Of Biomolecular Interactions*,” Biochem. Soc. Trans. 27(2):335-340; Malmqvist, M. *et al.* (1997) “*Biomolecular Interaction Analysis: Affinity Biosensor Technologies For Functional Analysis Of Proteins*,” Curr. Opin. Chem. Biol. 1(3):378-383; Fivash, M. *et al.* (1998) “*Biacore For Macromolecular*

Interaction,” Curr. Opin. Biotechnol. 9(1):97-101; Malmborg, A.C. *et al.* (1995) “*Biacore As A Tool In Antibody Engineering,*” *J. Immunol. Methods.* 183(1):7-13). The on- and off-rates of the Binding Domains of the Binding Molecules of the present invention can be readily altered by random or directed mutagenesis of nucleic acid molecules that encode such Binding Domains, followed by the routine screening of recovered nucleic acid molecules for their ability to encode mutated proteins that exhibit such altered binding kinetics.

[0083] The Binding Domains of the the Tri-Specific Binding Molecules of the present invention bind to epitopes in an “**immunospecific**” manner. As used herein, an antibody, diabody or other epitope binding molecule is said to “**immunospecifically**” bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and /or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means “specific” binding. Two molecules are said to be capable of binding to one another in a “**physiospecific**” manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0084] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (*i.e.*, more than two binding sites) for the same epitope and/or antigen.

[0085] Thus, in their simplest embodiment, the preferred binding molecules of the present invention are at least trispecific. Significantly, such molecules have at least

three Sites that are capable of binding antigen: an “**external**” Diabody-Type Binding Domain that is located furthest from Binding Domain III, an “internal” Diabody-Type Binding Domain that is located nearest to Binding Domain III, and Binding Domain III itself. The positions of such Domains are respectively designated as “**Site A**,” **Site B**” and “**Site C**” (**Figures 4A-4G**; **Figures 5A-5E**).

[0086] The Tri-Specific Binding Molecules of the present invention are able to coordinately bind to three different epitopes by comprising three binding domains. Two of the binding domains of such molecules are capable of binding to epitopes of “**Cancer Antigens**,” such that the molecule is capable of binding to two different Cancer Antigens. The third binding domain of such molecules is capable of binding to an epitope of a molecule expressed on the surface of an immune system effector cell (*i.e.*, an “**Effector Cell Antigen**”). Thus, the Tri-Specific Binding Molecules of the present invention are able to mediate coordinated and simultaneous binding to a cancer cell expressing two Cancer Antigens and to an immune system effector cell expressing the **Effector Cell Antigen**. The epitopes recognized by the Tri-Specific Binding Molecules of the present invention may be continuous or discontinuous (*e.g.*, conformational).

[0087] The first and second Cancer Antigens that are bound by the Cancer Antigen-Binding Domains of the trispecific binding molecules of the present invention may be selected from any molecule that is characteristically present on the surface of a cancer cell. One aspect of the present invention relates to the ability to target “**Low Expression Cancer Antigens**” (*i.e.*, a Cancer Antigen that may be expressed on a cancer cell at a level too low to permit a monospecific binding molecule to provide an effective cancer therapy). In contrast to such monospecific binding molecules, the Tri-Specific Binding Molecules of the present invention, by targeting two Cancer Antigens instead of one, exhibit synergistic and cooperative enhanced binding avidity that may compensate for low affinity of binding and thus may be advantageously used to target cancers characterized even by a Low Expression Cancer Antigen. A second aspect of the present invention relates to the ability to target “**Low Specificity Cancer Antigens**” (*i.e.*, a Cancer Antigen that may be expressed on a normal cell in addition to being expressed on a cancer cell). The Tri-Specific Binding Molecules of the

present invention, by providing synergistic and cooperative enhanced binding avidity to two Cancer Antigens, exhibits higher avidity of binding even for Low Specificity Cancer Antigens and thus provides a means for treating cancers that are characterized by such Cancer Antigens. Thus, the Tri-Specific Binding Molecules of the present invention may be used to impart an anti-cancer therapy even in circumstances where one or both of the target Cancer Antigens is ineffective on its own to provide such therapy.

[0088] For example, CD32B (the FcγRIIB receptor) is widely expressed on hematopoietic cells, including monocytes, macrophages, B cells, NK cells, neutrophils, mast cells, and platelets. Upon binding to IgG Fc Domain, CD32B inhibits the host immune system to thereby depress an ongoing immune response. Although such inhibition is desirable in helping the host recover from inflammatory reactions, it serves to exacerbate the immune deficiencies of subjects suffering from cancer or infectious disease. Antibodies that bind to CD32B, so as to block the binding of IgG Fc molecules, serve to prevent such inhibition and thus have utility as adjunct molecules in the treatment of cancer and infectious disease (Veri, M.C. *et al.* (2007) “*Monoclonal Antibodies Capable Of Discriminating The Human Inhibitory Fcγ-Receptor IIB (CD32B) From The Activating Fcγ-Receptor IIA (CD32A): Biochemical, Biological And Functional Characterization*,” *Immunology* 121(3):392-404). Unfortunately, CD32B is also expressed on liver sinusoidal endothelial cells (“LSE cells”) (Shahani, T. *et al.* (2014) “*Human Liver Sinusoidal Endothelial Cells But Not Hepatocytes Contain Factor VIII*,” *J. Thromb. Haemost.* 12(1):36-42; Géraud, C. *et al.* (2013) “*Endothelial Transdifferentiation In Hepatocellular Carcinoma: Loss Of Stabilin-2 Expression In Peri-Tumourous Liver Correlates With Increased Survival*,” *Liver Int.* 33(9):1428-1440; Takabe, Y. *et al.* (2012) “*Immunomagnetic Exclusion Of E-Cadherin-Positive Hepatoblasts In Fetal Mouse Liver Cell Cultures Impairs Morphogenesis And Gene Expression Of Sinusoidal Endothelial Cells*,” *J. Anat.* 221(3):229-239). Thus, antibodies that bind CD32B attack LSE cells. However, by forming a Tri-Specific Binding Molecule of the present invention that binds to CD32B and to antigens (*i.e.*, the first and second Cancer Antigens) that are not expressed on LSE cells, or are expressed at low levels by such cells (*i.e.*, Low Expression Cancer Antigen(s)), or are expressed with low

specificity on cancer cells and such LSE cells (*i.e.*, Low Specificity Cancer Antigen(s)), the present invention provides compositions and methods that would be used to depress CD32B-mediated immune system inhibition.

B. Exemplary Cancer Antigen-Binding Domains

[0089] Examples of suitable Cancer Antigens include: **19.9** as found in colon cancer, gastric cancer mucins; **4.2**; **A33** (a colorectal carcinoma antigen; Almquist, Y. 2006, *Nucl Med Biol.* Nov;33(8):991-998); **ADAM-9** (United States Patent Publication No. 2006/0172350; PCT Publication No. WO 06/084075); **AH6** as found in gastric cancer; **ALCAM** (PCT Publication No. WO 03/093443); **APO-1 (malignant human lymphocyte antigen)** (Trauth *et al.* (1989) "Monoclonal Antibody-Mediated Tumor Regression By Induction Of Apoptosis," *Science* 245:301-304); **B1** (Egloff, A.M. *et al.* 2006, *Cancer Res.* 66(1):6-9); **BAGE** (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **B7-H3**; **beta-catenin** (Prange W. *et al.* 2003 *J Pathol.* 201(2):250-9); **blood group ALe^b/Le^y** as found in colonic adenocarcinoma; **Burkitt's lymphoma antigen-38.13**, **C14** as found in colonic adenocarcinoma; **CA125 (ovarian carcinoma antigen)** (Bast, R.C. Jr. *et al.* 2005 *Int J Gynecol Cancer* 15 Suppl 3:274-81 ; Yu *et al.* (1991) "Coexpression Of Different Antigenic Markers On Moieties That Bear CA 125 Determinants," *Cancer Res.* 51(2):468-475); **Carboxypeptidase M** (United States Patent Publication No. 2006/0166291); **CD5** (Calin, G.A. *et al.* 2006 *Semin Oncol.* 33(2):167-73; **CD19** (Ghetie *et al.* (1994) "Anti-CD19 Inhibits The Growth Of Human B-Cell Tumor Lines In Vitro And Of Daudi Cells In SCID Mice By Inducing Cell Cycle Arrest," *Blood* 83:1329-1336; Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CD20** (Thomas, D.A. *et al.* 2006 *Hematol Oncol Clin North Am.* 20(5):1125-36); **CD22** (Kreitman, R.J. 2006 *AAPS J.* 18;8(3):E532-51); **CD23** (Rosati, S. *et al.* 2005 *Curr Top Microbiol Immunol.* 5;294:91-107); **CD25** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CD27** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD28** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD33** (Sgouros *et al.* (1993) "Modeling And Dosimetry Of Monoclonal Antibody M195 (Anti-CD33) In Acute Myelogenous Leukemia," *J. Nucl. Med.* 34:422-430); **CD36** (Ge, Y. 2005 *Lab Hematol.* 11(1):31-7); **CD40/CD154** (Messmer, D. *et al.* 2005 *Ann N Y Acad Sci.*

1062:51-60); **CD45** (Jurcic, J.G. 2005 *Curr Oncol Rep.* 7(5):339-46); **CD56** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD46** (United States Patent No. 7,148,038; PCT Publication No. WO 03/032814); **CD79a/CD79b** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48; Chu, P.G. *et al.* 2001 *Appl Immunohistochem Mol Morphol.* 9(2):97-106); **CD103** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CDK4** (Lee, Y.M. *et al.* 2006 *Cell Cycle* 5(18):2110-4); **CEA (carcinoembryonic antigen)** (Foon *et al.* (1995) "Immune Response To The Carcinoembryonic Antigen In Patients Treated With An Anti-Idiotypic Antibody Vaccine," *J. Clin. Invest.* 96(1):334-42); **CEA** (carcinoembryonic antigen; Mathelin, C. 2006 *Gynecol Obstet Fertil.* 34(7-8):638-46; Tellez-Avila, F.I. *et al.* 2005 *Rev Invest Clin.* 57(6):814-9); **CO17-1A** (Ragnhammar *et al.* (1993) "Effect Of Monoclonal Antibody 17-1A And GM-CSF In Patients With Advanced Colorectal Carcinoma - Long-Lasting, Complete Remissions Can Be Induced," *Int. J. Cancer* 53:751-758); **CO-43** (blood group Le^b); **CO-514** (blood group Le^a) as found in adenocarcinoma; **CTA-1**; **CTLA4** (Peggs, K.S. *et al.* 2006 *Curr Opin Immunol.* 18(2):206-13); **Cytokeratin 8** (PCT Publication No. WO 03/024191); **D1.1**; **D156-22**; **DR5** (Abdulghani, J. *et al.* (2010) "TRAIL Receptor Signaling And Therapeutics," *Expert Opin. Ther. Targets* 14(10):1091-1108; Andera, L. (2009) "Signaling Activated By The Death Receptors Of The TNFR Family," *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.* 153(3):173-180; Carlo-Stella, C. *et al.* (2007) "Targeting TRAIL Agonistic Receptors for Cancer Therapy," *Clin. Cancer* 13(8):2313-2317; Chaudhari, B.R. *et al.* (2006) "Following the TRAIL to Apoptosis," *Immunologic Res.* 35(3):249-262); **E₁ series** (blood group B) as found in pancreatic cancer; **EGFR (Epidermal Growth Factor Receptor)** (Adenis, A. *et al.* 2003 *Bull Cancer.* 90 Spec No:S228-32); **Ephrin receptors** (and in particular **EphA2** (United States Patent No. 7,569,672; PCT Publication No. WO 06/084226); **Erb** (ErbB1; ErbB3; ErbB4; Zhou, H. *et al.* 2002 *Oncogene* 21(57):8732-8740; Rimon, E. *et al.* 2004 *Int J Oncol.* 24(5):1325-1338); **GAGE** (GAGE-1; GAGE-2; Akcakanat, A. *et al.* 2006 *Int J Cancer.* 118(1):123-128); **GD2/GD3/GM2** (Livingston, P.O. *et al.* 2005 *Cancer Immunol Immunother.* 54(10):1018-1025); **F3** as found in lung adenocarcinoma; **FC10.2** as found in embryonal carcinoma cells and gastric adenocarcinoma; **G49, ganglioside GD2** (Saleh *et al.* (1993) "Generation Of A

Human Anti-Idiotypic Antibody That Mimics The GD2 Antigen,” J.Immunol., 151, 3390-3398); **ganglioside GD3** (Shitara *et al.* (1993) “A Mouse/Human Chimeric Anti-(Ganglioside GD3) Antibody With Enhanced Antitumor Activities,” Cancer Immunol. Immunother. 36:373-380); **ganglioside GM2** (Livingston *et al.* (1994) “Improved Survival In Stage III Melanoma Patients With GM2 Antibodies: A Randomized Trial Of Adjuvant Vaccination With GM2 Ganglioside,” J. Clin. Oncol. 12:1036-1044); **ganglioside GM3** (Hoon *et al.* (1993) “Molecular Cloning Of A Human Monoclonal Antibody Reactive To Ganglioside GM3 Antigen On Human Cancers,” Cancer Res. 53:5244-5250); **G_{D2}**; **G_{D3}**; **GICA 19-9** (Herlyn *et al.* (1982) “Monoclonal Antibody Detection Of A Circulating Tumor-Associated Antigen. I. Presence Of Antigen In Sera Of Patients With Colorectal, Gastric, And Pancreatic Carcinoma,” J. Clin. Immunol. 2:135-140); **G_{M2}**; **gp100** (Lotem, M. *et al.* 2006 *J Immunother.* 29(6):616-27); **Gp37 (human leukemia T cell antigen)** (Bhattacharya-Chatterjee *et al.* (1988) “Idiotypic Vaccines Against Human T Cell Leukemia. II. Generation And Characterization Of A Monoclonal Idiotypic Cascade (Ab1, Ab2, and Ab3),” J. Immunol. 141:1398-1403); **gp75 (melanoma antigen)** (Vijayasardahl *et al.* (1990) “The Melanoma Antigen Gp75 Is The Human Homologue Of The Mouse B (Brown) Locus Gene Product,” J. Exp. Med. 171(4):1375-1380); **gpA33**; **HER2 antigen (p185^{HER2})** (Kumar, Pal S *et al.* 2006 *Semin Oncol.* 33(4):386-91); **human B-lymphoma antigen-CD20** (Reff *et al.* (1994) “Depletion Of B Cells In Vivo By A Chimeric Mouse Human Monoclonal Antibody To CD20,” Blood 83:435-445); **human milk fat globule antigen**; **human papillomavirus-E6/human papillomavirus-E7** (DiMaio, D. *et al.* 2006 *Adv Virus Res.* 66:125-59); **HMW-MAA (high molecular weight melanoma antigen)** (Natali *et al.* (1987) “Immunohistochemical Detection Of Antigen In Human Primary And Metastatic Melanomas By The Monoclonal Antibody 140.240 And Its Possible Prognostic Significance,” Cancer 59:55-63; Mittelman *et al.* (1990) “Active Specific Immunotherapy In Patients With Melanoma. A Clinical Trial With Mouse Antiidiotypic Monoclonal Antibodies Elicited With Syngeneic Anti-High-Molecular-Weight-Melanoma-Associated Antigen Monoclonal Antibodies,” J. Clin. Invest. 86:2136-2144); **I antigen (differentiation antigen)** (Feizi (1985) “Demonstration By Monoclonal Antibodies That Carbohydrate Structures Of Glycoproteins And Glycolipids Are Onco-Developmental Antigens,” Nature 314:53-57) such as **I(Ma)** as

found in gastric adenocarcinomas; **Integrin Alpha-V-Beta-6** (PCT Publication No. WO 03/087340); **JAM-3** (PCT Publication No. WO 06/084078); **KID3** (PCT Publication No. WO 05/028498); **KID31** (PCT Publication No. WO 06/076584); **KS 1/4 pan-carcinoma antigen** (Perez *et al.* (1989) "Isolation And Characterization Of A cDNA Encoding The Ks1/4 Epithelial Carcinoma Marker," J. Immunol. 142:3662-3667; Möller *et al.* (1991) "Bi-specific-Monoclonal-Antibody-Directed Lysis Of Ovarian Carcinoma Cells By Activated Human T Lymphocytes," Cancer Immunol. Immunother. 33(4):210-216; Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); **L6 and L20 (human lung carcinoma antigens)** (Hellström *et al.* (1986) "Monoclonal Mouse Antibodies Raised Against Human Lung Carcinoma," Cancer Res. 46:3917-3923); **LEA**; **LUCA-2** (United States Patent Publication No. 2006/0172349; PCT Publication No. WO 06/083852); **M1:22:25:8**; **M18**; **M39**; **MAGE** (MAGE-1; MAGE-3; (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **MART** (Kounalakis, N. *et al.* 2005 *Curr Oncol Rep.* 7(5):377-82; **MUC-1** (Mathelin, C. 2006 *Gynecol Obstet Fertil.* 34(7-8):638-46); **MUM-1** (Castelli, C. *et al.* 2000 *J Cell Physiol.* 182(3):323-31); **Myl**; **N-acetylglucosaminyltransferase** (Dennis, J.W. 1999 *Biochim Biophys Acta.* 6;1473(1):21-34); **neoglycoprotein**; **NS-10** as found in adenocarcinomas; **OFA-1**; **OFA-2**; **Oncostatin M** (Oncostatin Receptor Beta) (United States Patent No. 7,572,896; PCT Publication No. WO 06/084092); **p15** (Gil, J. *et al.* 2006 *Nat Rev Mol Cell Biol.* 7(9):667-77); **p97 (melanoma-associated antigen)** (Estin *et al.* (1989) "Transfected Mouse Melanoma Lines That Express Various Levels Of Human Melanoma-Associated Antigen p97," J. Natl. Cancer Instit. 81(6):445-454); **PEM (polymorphic epithelial mucin)** (Hilkens *et al.* (1992) "Cell Membrane-Associated Mucins And Their Adhesion-Modulating Property," Trends in Biochem. Sci. 17:359-363); **PEMA (polymorphic epithelial mucin antigen)**; **PIPA** (United States Patent No. 7,405,061; PCT Publication No. WO 04/043239); **PSA (prostate-specific antigen)** (Henttu *et al.* (1989) "cDNA Coding For The Entire Human Prostate Specific Antigen Shows High Homologies To The Human Tissue Kallikrein Genes," Biochem. Biophys. Res. Comm. 10(2):903-910; Israeli *et al.* (1993) "Molecular Cloning Of A Complementary DNA Encoding A Prostate-Specific Membrane Antigen," Cancer Res. 53:227-230; Cracco, C.M. *et al.* 2005 *Minerva Urol Nefrol.* 57(4):301-11); **PSMA (prostate-specific membrane antigen)**

(Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-180); **prostatic acid phosphate** (Tailor *et al.* (1990) "Nucleotide Sequence Of Human Prostatic Acid Phosphatase Determined From A Full-Length cDNA Clone," *Nucl. Acids Res.* 18(16):4928); **R₂₄** as found in melanoma; **ROR1** (United States Patent No. 5,843,749); **sphingolipids**; **SSEA-1**; **SSEA-3**; **SSEA-4**; **sTn** (Holmberg, L.A. 2001 *Expert Opin Biol Ther.* 1(5):881-91); **T cell receptor derived peptide** from a cutaneous T cell lymphoma (see Edelson (1998) "Cutaneous T-Cell Lymphoma: A Model For Selective Immunotherapy," *Cancer J Sci Am.* 4:62-71); **T₅A₇** found in myeloid cells; **TAG-72** (Yokota *et al.* (1992) "Rapid Tumor Penetration Of A Single-Chain Fv And Comparison With Other Immunoglobulin Forms," *Cancer Res.* 52:3402-3408); **TL5** (blood group A); **TNF-receptor** (TNF- α receptor, TNF- β receptor; **TNF- γ receptor** (van Horssen, R. *et al.* 2006 *Oncologist.* 11(4):397-408; Gardnerova, M. *et al.* 2000 *Curr Drug Targets.* 1(4):327-64); **TRA-1-85** (blood group H); **Transferrin Receptor** (United States Patent No. 7,572,895; PCT Publication No. WO 05/121179); **TSTA (tumor-specific transplantation antigen)** such as virally-induced tumor antigens including T-antigen DNA tumor viruses and envelope antigens of RNA tumor viruses, oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (Hellström *et al.* (1985) "Monoclonal Antibodies To Cell Surface Antigens Shared By Chemically Induced Mouse Bladder Carcinomas," *Cancer. Res.* 45:2210-2188); **VEGF** receptor (O'Dwyer. P.J. 2006 *Oncologist.* 11(9):992-998); **VEP8**; **VEP9**; **VIM-D5**; and **Y hapten, Le^y** as found in embryonal carcinoma cells.

1. Campath-1 (CD52) Binding Domain (Alemtuzumab)

[0090] The amino acid sequence of the VL Domain of the humanized anti-CD52 antibody "**Alemtuzumab**" (SEQ ID NO:205) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVITCKASQNID KYLNWYQQKP GKAPKLLIYN
TNNLQTGVPS RFSGSGSGTD FTFTISSLQP EDIATYYCLQ HISRPRTFGQ
 GTKVEIKR

[0091] The amino acid sequence of the VH Domain of the humanized anti-CD52 antibody “Alemtuzumab” (SEQ ID NO:206) is shown below (CDR residues are shown underlined):

QVQLQESGPG LVRPSQTLST TCTVSGFTFT DFYMNWVRQP PGRGLEWIGF
IRDKAKGYTT EYNPSVKGRV TMLVDTSKNQ FSLRLSSVTA ADTAVYYCAR
EGHTAAPFDY WGQGSLVTVS S

2. CD317 (BMST2)-Binding Domains

[0092] CD317 (also known as Bone Marrow Stromal Cell Antigen 2; BMST) is overexpressed on various cancer cells isolated from breast, lung, kidney, endometrium, and skin (Kawai, S. *et al.* (2008) “Interferon- α enhances CD317 expression and the antitumor activity of anti-CD317 monoclonal antibody in renal cell carcinoma xenograft models,” *Cancer Science* 99(12):2461-2466; Cai, D. *et al.* (2009) “Up-Regulation Of Bone Marrow Stromal Protein 2 (BST2) In Breast Cancer With Bone Metastasis,” *BMC Cancer* 9:102, pp. 1-10; Wang, W. *et al.* (2009) *HM1.24 (CD317) Is A Novel Target Against Lung Cancer For Immunotherapy Using Anti-HM1.24 Antibody*,” *Cancer Immunology, Immunotherapy* 58(6):967-976; Wang, W. *et al.* (2009) “Chimeric And Humanized Anti-HM1.24 Antibodies Mediate Antibody-Dependent Cellular Cytotoxicity Against Lung Cancer Cells. *Lung Cancer*,” 63(1):23-31; Sayeed, A. *et al.* (2013) “Aberrant Regulation Of The BST2 (Tetherin) Promoter Enhances Cell Proliferation And Apoptosis Evasion In High Grade Breast Cancer Cells,” *PLoS ONE* 8(6):e67191, pp. 1-10; Yi, E.H. *et al.* (2013) “BST-2 Is A Potential Activator Of Invasion And Migration In Tamoxifen-Resistant Breast Cancer Cells,” *Biochem. Biophys. Res. Commun.* 435(4):685-690; Staudinger, M. (2014) “The Novel Immunotoxin HM1.24-ETA' Induces Apoptosis In Multiple Myeloma Cells,” *Blood Cancer J.* 13;4:e219, pp. 1-11). Antibodies that immunospecifically bind to CD317 are commercially available (Novus Biologicals LLC; BioLegend, Inc.; see also U.S. Patent No. 8,834,876, which references the deposit of the heavy and light chains of antibody HM1.24 as FERM BP-5644 and FERM BP-5646; see also U.S. Patent No. 8,394,374). The amino acid sequence of the VL Domain of the anti-CD317 antibody “HM1.24” (SEQ ID NO:302) is shown below (CDR residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITCKKASQDV NTAVAWYQQK PGQSPKLLIY
SASNRYTGVP DRITGSGSGT DFTFTISSVQ AEDLALTTCQ QHYSTPFTFG
 SGTKEIK

[0093] The amino acid sequence of the VH Domain of the anti-CD317 antibody “HM1.24” (SEQ ID NO:303) is shown below (CDR residues are shown underlined):

QVQLQQSGAE LARPGASVKL SCKASGYTFT PYWMQWVKQR PGQGLEWIGS
IFPGDGDTRY SQKFKGKATL TADKSSSTAY MQLSILAFED SAVYYCARGL
RRGGYYFDYW GQGTTTLTVSS

3. CEACAM5- and CEACAM6-Binding Domains

[0094] Carcinoembryonic Antigen-Related Cell Adhesion Molecules 5 (CEACAM5) and 6 (CEACAM6) have been found to be associated with various types of cancers including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660), and particularly colorectal, gastrointestinal, pancreatic, non-small cell lung cancer (NSCL), breast, thyroid, stomach, ovarian and uterine carcinomas (Zheng, C. *et al.* (2011) “A Novel Anti-CEACAM5 Monoclonal Antibody, CC4, Suppresses Colorectal Tumor Growth and Enhances NK Cells-Mediated Tumor Immunity,” PLoS One 6(6):e21146, pp. 1-11).

[0095] CEACAM5 has been found to be overexpressed in 90% of gastrointestinal, colorectal and pancreatic cancers, 70% of non-small cell lung cancer cells and 50% of breast cancers (Thompson, J.A. *et al.* (1991) “Carcinoembryonic Antigen Gene Family: Molecular Biology And Clinical Perspectives,” J. Clin. Lab. Anal. 5:344-366).

[0096] Overexpressed carcinoembryonic antigen-related cellular adhesion molecule 6 (CEACAM6) plays important roles in the invasion and metastasis of a variety of human cancers, including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660; Deng, X. *et al.* (2014) “Expression Profiling Of

CEACAM6 Associated With The Tumorigenesis And Progression In Gastric Adenocarcinoma,” Genet. Mol. Res. 13(3):7686-7697; Cameron, S. *et al.* (2012) “*Focal Overexpression Of CEACAM6 Contributes To Enhanced Tumourigenesis In Head And Neck Cancer Via Suppression Of Apoptosis,*” Mol. Cancer 11:74, pp. 1-11; Chapin, C. *et al.* (2012) “*Distribution And Surfactant Association Of Carcinoembryonic Cell Adhesion Molecule 6 In Human Lung,*” Amer. J. Physiol. Lung Cell. Mol. Physiol. 302(2):L216-L25; Riley, C.J. *et al.* (2009) “*Design And Activity Of A Murine And Humanized Anti-CEACAM6 Single-Chain Variable Fragment In The Treatment Of Pancreatic Cancer,*” Cancer Res. 69(5):1933-1940; Lewis-Wambi, J.S. *et al.* (2008) “*Overexpression Of CEACAM6 Promotes Migration And Invasion Of Oestrogen-Deprived Breast Cancer Cells,*” Eur. J. Cancer 44(12):1770-1779; Blumenthal, R.D. *et al.* (2007) “*Expression Patterns Of CEACAM5 And CEACAM6 In Primary And Metastatic Cancers,*” BMC Cancer. 7:2, pp. 1-15). Antibodies that immunospecifically bind to CEACAM5 and CEACAM6 are commercially available (Santa Cruz Biotechnology, Inc., Novus Biologicals LLC; Abnova Corporation). The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / ANTI-CEACAM6 antibody 16C3 (EP 2585476) (SEQ ID NO:304) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCGASENIY GALNWYQRKP GKSPKLLIWG
ASNLADGMPS RFGSGSGRQ YTLTISSLQP EDVATYYCQN VLSSPYTFGG
 GTKLEIK

[0097] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / ANTI-CEACAM6 antibody 16C3 (EP 2585476) (SEQ ID NO:305) is shown below (CDR residues are shown underlined):

QVQLQQSGPE VVRPGVSVKI SCKGSGYTFT DYAMHWVKQS HAKSLEWIGL
ISTYSGDTKY NQNFKGKATM TVDKSASTAY MELSSLRSED TAVYYCARGGD
YSGSRYWFAY WGQGTLLVTVS S

[0098] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / CEACAM6 antibody hMN15 (WO 2011/034660) (SEQ ID NO:306) is shown below (CDR residues are shown underlined):

DIQLTQSPSS LSASVGDRVT MTCSASSRVS YIHWYQQKPG KAPKRWIYGT
STLASGVPAR FSGSGSGTDF TFTISSLQPE DIATYYCQQW SYNPPTFGQG
 TKVEIKR

[0099] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / CEACAM6 antibody hMN15 (WO 2011/034660) (**SEQ ID NO:307**) is shown below (CDR residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCSSSGFALT DYYMSWVRQA PGKGLEWLGF
IANKANGHTT DYSPSVKGRF TISRDN SKNT LFLQMDSL RP EDTGVYFCAR
DMGIRWNFDV WGQGTPVTVS S

4. DR5-Binding Domains

[00100] DR5 is a preferred Cancer Antigen of the present invention. The preferred anti-human DR5-binding molecules of the present invention possess the VL and/or VH Domains of murine anti-human DR5 monoclonal antibodies “**DR5 mAb 1**” and/or “**DR5 mAb 2**,” and more preferably possess 1, 2 or all 3 of the CDRs of the VL Domain and/or 1, 2 or all 3 of the CDRs of the VH Domain of such anti-human DR5 monoclonal antibodies. Alternatively, any anti-human DR5 monoclonal antibody may be employed, particularly: **drozitumab** (designated herein as “**DR5 mAb 3**”), **conatumumab** (designated herein as “**DR5 mAb 4**”), **tigatumumab** (designated herein as “**DR5 mAb 5**”), **LBY135-1** (designated herein as “**DR5 mAb 6**”), **LBY135-2** (designated herein as “**DR5 mAb 7**”) and **KMTR2** (designated herein as “**DR5 mAb 8**”).

a. The Anti-Human DR5 Antibody DR5 mAb 1

[00101] DR5 has potential utility in the treatment of a wide range of cancers (*e.g.*, colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin’s lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer. The amino acid sequence of human DR5 precursor (NCBI Sequence NP_003833.4) (**SEQ ID NO:2**) is:

MEQRGQNAPA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAAVLLLLVS
 AESALITQQD LAPQQRVAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
 QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
 EMCRCRGTGC PRGMVKVGDC TPWSDIECVH KESGTKHSGE APAVEETVTS
 SPGTPASPCS LSGIIIGVTV AAVVLIVAVF VCKSLLWKKV LPYLKGICSG
 GGGDPERVDR SSQRPGAEDN VLNEIVSILQ PTQVPEQEME VQEPAEPTGV
 NMLSPGESEH LLEPAEAERS QRRRLLPAN EGDPTETLRQ CFDDFADLVP
 FDSWEPLMRK LGLMDNEIKV AKAEAAGHRD TLYTMLIKWV NKTGRDASVH
 TLLDALETLG ERLAKQKIED HLLSSGKFMY LEGNADSAMS

[00102] The amino acid sequence of the VL Domain of DR5 mAb 1 (SEQ ID NO:3) is shown below (CDR residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI K

CDR_L1 of DR5 mAb 1 (SEQ ID NO:4): **RASKSVSSSGYSYMH**

CDR_L2 of DR5 mAb 1 (SEQ ID NO:5): **LSSNLDS**

CDR_L3 of DR5 mAb 1 (SEQ ID NO:6): **QHSRDLPP**

[00103] The VL Domain of DR5 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:7) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gacattgtgc tgacacagtc tcttgcttc ttagctgtat ctctcgggca
gagggccacc atctcatgca agggccagcaa aagtgtcagt tcctctggct
atagttatat gcactggtag caacagaaac caggacagcc acccaaagtc
ctcatctttc c ttccatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggt tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgt cagc acagtaggga tcttcctccg
acgttcgggtg gaggcaccaa gctggaaatc aaa

[00104] The amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8) is shown below (CDR residues are shown underlined). The C-terminal amino acid may be substituted with alanine to facilitate subcloning of this VH Domain.

EVKFLESGGG LVQPGGSLKL SCVASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTRRA
YYGNPAWFAY WGQGTTLVTVSS

CDR_H1 of DR5 mAb 1 (SEQ ID NO:9): **GFDFSRYWMS**

CDR_H2 of DR5 mAb 1 (SEQ ID NO:10): **EINPDSNTINYTPSLKD**

CDR_H3 of DR5 mAb 1 (SEQ ID NO:11): **RAYGNPAWFAY**

[00105] The VH Domain of DR5 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:12) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tctgtgtag cctcagatt cgattttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggaggaa
attaatccag atagcaatac gataaactat acgccatctc taaaggataa

attcatcatc tccagagaca acgccccaaa tacgctgtat ctgcaaatga
 ccaaagtgag atctgaggac acagcccttt attattgtac aagaagggcc
tactatggta acccggcctg gtttgettac tggggccaag ggactctggt
 cactgtctct tcc

b. The Anti-Human DR5 Antibody DR5 mAb 2

(1) Murine Anti-Human Antibody DR5 mAb 2

[00106] The amino acid sequence of the VL Domain of DR5 mAb 2 (SEQ ID NO:13) is shown below (CDR residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVDPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
 GTKLEIK

CDR_{L1} of DR5 mAb 2 (SEQ ID NO:14): **KASQDVNTAVA**

CDR_{L2} of DR5 mAb 2 (SEQ ID NO:15): **WASTRHT**

CDR_{L3} of DR5 mAb 2 (SEQ ID NO:16): **QQHYITPWT**

[00107] The VL Domain of DR5 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:17) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gacattgtga tgaccagtc tcacaaattc atgtccactt cagtaggaga
 cagggtcagc atcacctgca a agggcagtcaggatgtgaat actgctgtag
cctgggtatca acaaaaacca gggcaatctc ctaaaactact gatttactggg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
 tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
 cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
 ggcaccaagc tggaaatcaaa

[00108] The amino acid sequence of the VH Domain of DR5 mAb 2 (SEQ ID NO:18) is shown below (CDR residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKASGYTFT EYILHWVKQK SGQGLEWIGW
FYPGNNNIKY NEKFKD KATL TADKSSSTVY MELSRITSED SAVYFCARHE
QGPGYFDYWG QGTTLTVSS

CDR_{H1} of DR5 mAb 2 (SEQ ID NO:19): **GYTFTEYILH**

CDR_{H2} of DR5 mAb 2 (SEQ ID NO:20): **WFYPGNNNIKYNEKF**KD

CDR_{H3} of DR5 mAb 2 (SEQ ID NO:21): **HEQGPGYFDY**

[00109] The VH Domain of DR5 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:22) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

```
aaggtccagc tgcagcagtc tggagctgaa ctggtgaaac ccggggcatc
agtgaagctg tcctgcaagg cttctgggta caccttcact gagtatattt
tacactgggt aaagcagaag tctggacagg gtcttgagtg gattgggttgg
ttttatcctg gaaataataa tataaagtac aatgagaaat tcaaggacaa
ggccacactg actgcggaca aatcctccag cacagtctat atggaactta
gtagattgac atctgaagac tctgcggtct atttctgtgc aagacacgaa
caaggaccag gttactttga ctactggggc caaggcacca ctctcacagt
ctcctcc
```

(2) Humanized DR5 mAb 2 (“hDR5 mAb 2”)

[00110] The above-described murine anti-human DR5 antibody DR5 mAb 2 was humanized in order to demonstrate the capability of humanizing an anti-human DR5 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded four humanized VL Domains designated herein as “**hDR5 mAb 2 VL-2**,” “**hDR5 mAb 2 VL-3**,” “**hDR5 mAb 2 VL-4**,” and “**hDR5 mAb 2 VL-5**,” and one humanized VH Domain, designated herein as “**hDR5 mAb 2 VH-2**.” Any of the humanized VL Domains may be paired with the humanized VH Domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as “**hDR5 mAb 2**,” and particular combinations of humanized VL/VH Domains are referred to by reference to the VL domain.

[00111] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-2** (SEQ ID NO:23) is shown below (CDR residues are shown underlined):

```
DIQMTQSPSF LSASVGDRVT ITCKASQDVN TAVAWYQQK GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIK
```

[00112] **hDR5 mAb 2 VL-2** is preferably encoded by a polynucleotide (SEQ ID NO:24) having the sequence shown below:

```
gatattcaga tgaccacagag tccctcatTT ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgta aagcttctca ggatgtcaac accgccgtgg
cttgggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccatct aggttctctg gcagtggatc
agggacagac tttaacctga caattagctc cctgcagccc gaggatgtgg
```

ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
ggcacaaaac tggaaatcaa a

[00113] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-3 (SEQ ID NO:25)** is shown below (CDR residues are shown underlined):

DIQMTQSPSF LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVDP RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIK

[00114] **hDR5 mAb 2 VL-3** is preferably encoded by a polynucleotide (**SEQ ID NO:26**) having the sequence shown below:

gatattcaga tgacccagag tccctcatTT ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg
cttggtagca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
ggcacaaaac tggaaatcaa a

[00115] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-4 (SEQ ID NO:27)** is shown below (CDR residues are shown underlined):

DIQMTQSPSF LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIK

[00116] **hDR5 mAb 2 VL-4** is preferably encoded by a polynucleotide (**SEQ ID NO:28**) having the sequence shown below:

gatattcaga tgacccagag tccctcatTT ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg
cttggtagca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccactc aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagcca gaggatatcg
ctacatacta ttgtcagcag cactacatca ctccttggac cttcggcggg
ggcacaaaac tggaaatcaa a

[00117] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-5 (SEQ ID NO:29)** is shown below (CDR residues are shown underlined):

DIQMTQSPSF LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVDP RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIK

[00118] **hDR5 mAb 2 VL-5** is preferably encoded by a polynucleotide (SEQ ID NO:30) having the sequence shown below:

```
gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgcctggg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac tttaccctga caattagctc cctgcagccc gaggatatcg
ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcgagg
ggcacaaaac tggaaatcaa a
```

[00119] The amino acid sequence of the VH Domain of **hDR5 mAb 2 VH-2** (SEQ ID NO:31) is shown below (CDR residues are shown underlined):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT EYILHWVRQA PGQGLEWMGW
FYPGNNNIKY NEKFKDRVTI TADKSTSTVY MELSSLRSED TAVYYCARHE
QPGGYFDYWG QGTLVTVSS
```

[00120] **hDR5 mAb 2 VH-2** is preferably encoded by a polynucleotide (SEQ ID NO:32) having the sequence shown below:

```
caggtccagc tgggtgcagag tgggggcagag gtgaaaaagc caggggcatc
agtgaaagtg tcttgtaaag catcagggtta tacatttact gagtacatcc
tgactgggtg ggcacaggca ccaggacagg gactggaatg gatgggggtgg
ttctaccctg gcaacaacaa cattaagtac aacgagaagt ttaaagaccg
ggtgaccatc acagcggata agtctaccag tacagtctat atggagctga
gctccctgag aagcgaagac accgccgtct actattgcgc tcgccacgaa
caggggtccag gttactttga ttattggggg caggggaactc tggtcacagt
cagctcc
```

[00121] The CDR1 of the VL Domain of **hDR5 mAb 2 VL-3**, **hDR5 mAb 2 VL-4** and **hDR5 mAb VL-5** has the amino acid sequence: **RASQDVNTAVA** (SEQ ID NO:320).

c. **Drozitumab ("DR5 mAb 3")**

[00122] The amino acid sequence of the VL Domain of **drozitumab ("DR5 mAb 3")** (SEQ ID NO:54) is shown below (CDR residues are shown underlined):

```
SELTQDPAVS VALGQTVRIT CSGDSLRSYY ASWYQQKPG QAPVLVIYGA
NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSA DSSGNHVVFG
GGTKLTVLG
```

CDR_L1 of DR5 mAb 3 (SEQ ID NO:55): **SGDSLRSYYAS**

CDR_L2 of DR5 mAb 3 (SEQ ID NO:56): **GANNRPS**

CDR_L3 of DR5 mAb 3 (SEQ ID NO:57): **NSADSSGNHVV**

[00123] The amino acid sequence of the VH Domain of **drozitumab** (“**DR5 mAb 3**”) (SEQ ID NO:58) is shown below (CDR residues are shown underlined):

EVQLVQSGGG VERPGGSLRL SCAASGFTFD DYAMSWVRQA PGKGLEWVSG
INWQGGSTGY ADSVKGRVTI SRDNAKNSLY LQMNSLRAED TAVYYCAKIL
GAGRGWYFDY WGKGTTTVTS S

CDR_H1 of DR5 mAb 3 (SEQ ID NO:59): **GFTFD****DYAMS**

CDR_H2 of DR5 mAb 3 (SEQ ID NO:60): **INWQGGSTGYADSVKG**

CDR_H3 of DR5 mAb 3 (SEQ ID NO:61): **ILGAGRGWYFDY**

d. Conatumumab (“DR5 mAb 4”)

[00124] The amino acid sequence of the VL Domain of **conatumumab** (“**DR5 mAb 4**”) (SEQ ID NO:62) is shown below (CDR residues are shown underlined):

EIVLTQSPGT LSLSPGERAT LSCRASQGIS RSYLAWYQQK PGQAPSLLIY
GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QFGSSPWTFG
 QGTKVEIK

CDR_L1 of DR5 mAb 4 (SEQ ID NO:63): **RASQGIS****RSYLA**

CDR_L2 of DR5 mAb 4 (SEQ ID NO:64): **GASSRAT**

CDR_L3 of DR5 mAb 4 (SEQ ID NO:65): **QQFGSSPWT**

[00125] The amino acid sequence of the VH Domain of **conatumumab** (“**DR5 mAb 4**”) (SEQ ID NO:66) is shown below (CDR residues are shown underlined):

QVQLQESGPG LVKPSQTLST TCTVSGGGIS SGDYFWSWIR QLPKGLEWII
 GHIHNSGTTY YNPSLKSRVT ISVDTSKKQF SLRLSSVTAA DTAVYYCARDD
RGGDYYYGMD VWGQGTTTVT SS

CDR_H1 of DR5 mAb 4 (SEQ ID NO:67): **GGSISSGDYFWS**

CDR_H2 of DR5 mAb 4 (SEQ ID NO:68): **HIHNSGTTYYNPSLKS**

CDR_H3 of DR5 mAb 4 (SEQ ID NO:69): **DRGGDYYYGMDV**

e. Tigatumumab (“DR5 mAb 5”)

[00126] The amino acid sequence of the VL Domain of **tigatumumab** (“**DR5 mAb 5**”) (SEQ ID NO:70) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVIT ITCKASQDVG TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YSSYRTFGQG
 TKVEIK

CDR_L1 of DR5 mAb 5 (SEQ ID NO:71): **KASQDVGTAVA**

CDR_L2 of DR5 mAb 5 (SEQ ID NO:72): **WASTRHT**

CDR_L3 of DR5 mAb 5 (SEQ ID NO:73): **QQYSSYRT**

[00127] The amino acid sequence of the VH Domain of **tigatumumab** (“**DR5 mAb5**”) (SEQ ID NO:74) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAAS**GFTFS** **SYVMS**WVRQA PGKGLEWVAT
ISSGGSYTY **PDSVKGR**FTI SRDNAKNTLY LQMNSLRAED TAVYYCAR**RG**
DSMITTDYWG QGTLVTVSS

CDR_H1 of DR5 mAb 5 (SEQ ID NO:75): **GFTFSSYVMS**

CDR_H2 of DR5 mAb 5 (SEQ ID NO:76): **TISSGGSYTYYPDSVKG**

CDR_H3 of DR5 mAb 5 (SEQ ID NO:77): **RGDSMITTDY**

f. LBY135-1 (“DR5 mAb 6”)

[00128] The amino acid sequence of the VL Domain of **LBY135-1** (“**DR5 mAb 6**”) (SEQ ID NO:78) is shown below (CDR residues are shown underlined):

DIAMTQSHKF MSTLVGDRVS ITCKAS**QDVN** **TAIA**WYQQKP GQSPKLLIYW
ASTRHTGVPD RFYGSGSGTD YTLTISSMEA EDAATYYC**QQ** **WSSNPLT**FGA
 GTKLELKRA

CDR_L1 of DR5 mAb 6 (SEQ ID NO:79): **QDVNTAIA**

CDR_L2 of DR5 mAb 6 (SEQ ID NO:80): **WASTRHT**

CDR_L3 of DR5 mAb 6 (SEQ ID NO:81): **QQWSSNPLT**

[00129] The amino acid sequence of the VH Domain of **LBY135-1** (“**DR5 mAb 6**”) (SEQ ID NO:82) is shown below (CDR residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **DYTIH**WVKQR SGQGLEWIGW
FYPGGGYIKY **NEKFKD**RATL TADKSSNTVY MELSRLTSEG SAVYFCAR**HE**
EGIFYFDYWGQ GTTLTVSS

CDR_H1 of DR5 mAb 6 (SEQ ID NO:83): **GYTFTDYTIH**

CDR_H2 of DR5 mAb 6 (SEQ ID NO:84): **WFYPGGGYIKYNEKFKD**

CDR_H3 of DR5 mAb 6 (SEQ ID NO:85): **HEEGIFYFDY**

g. LBY135-2 (“DR5 mAb 7”)

[00130] The amino acid sequence of the VL Domain of **LBY135-2 (“DR5 mAb 7”)** (**SEQ ID NO:86**) is shown below (CDR residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAIAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTISSVQA EDLALYYCQQ HYTTPFTFGS
 GTKL

CDR_L1 of DR5 mAb 7 (**SEQ ID NO:87**): **KASQDVNTAIA**

CDR_L2 of DR5 mAb 7 (**SEQ ID NO:88**): **WASTRHT**

CDR_L3 of DR5 mAb 7 (**SEQ ID NO:89**): **QQHYTTPFT**

[00131] The amino acid sequence of the VH Domain of **LBY135-2 (“DR5 mAb 7”)** (**SEQ ID NO:90**) is shown below (CDR residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKASGYTFT DYTHWVKQR SGQGLEWIGW
FYPGGGYIKY NEKFKDRATL TADKSSNTVY MELSRLTSED SAVYFCARHE
EGIFYFDYWGQ GTTLTVSS

CDR_H1 of DR5 mAb 7 (**SEQ ID NO:91**): **GYTFTDYTIH**

CDR_H2 of DR5 mAb 7 (**SEQ ID NO:92**): **WFYPGGGYIKYNEKF**KD

CDR_H3 of DR5 mAb 7 (**SEQ ID NO:93**): **HEEGIFYFDY**

h. KMTR2 (“DR5 mAb 8”)

[00132] The amino acid sequence of the VL Domain of **KMTR2 (“DR5 mAb 8”)** (**SEQ ID NO:94**) is shown below (CDR residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFTGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGG
 GTKVEIKR

CDR_L1 of DR5 mAb 8 (**SEQ ID NO:95**): **RASQSVSSYLA**

CDR_L2 of DR5 mAb 8 (**SEQ ID NO:96**): **DASN**RAT

CDR_L3 of DR5 mAb 8 (**SEQ ID NO:97**): **QQRSNWPLT**

[00133] The amino acid sequence of the VH Domain of **KMTR2 (“DR5 mAb 8”)** (**SEQ ID NO:98**) is shown below (CDR residues are shown underlined):

QVQLVQSGAE MKKPGASVKV SCKTSGYTFT NYKINWVRQA PGQGLEWMGW
MNPDTDSTGY PQKFQGRVTM TRNTSISTAY MELSSLRSED TAVYYCARSY
SGSSYRDYY YGMDVWGQGT TVTVSS

CDR_H1 of DR5 mAb 8 (SEQ ID NO:99): **GYTFTNYKIN**
CDR_H2 of DR5 mAb 8 (SEQ ID NO:100): **WMNPDTDSTGY PQKFQG**
CDR_H3 of DR5 mAb 8 (SEQ ID NO:101): **SYGSGSYRDYYYGMDV**

5. EphA2-Binding Domains

[00134] The receptor tyrosine kinase, ephrin type-A receptor 2 (**EphA2**) is a preferred cancer antigen of the present invention. EphA2 is normally expressed at sites of cell-to-cell contact in adult epithelial tissues, however, recent studies have shown that it is also overexpressed in various types of epithelial carcinomas, with the greatest level of EphA2 expression observed in metastatic lesions. High expression levels of EphA2 have been found in a wide range of cancers and in numerous tumor cell lines, including prostate cancer, breast cancer, non-small cell lung cancer and melanoma (Xu, J. *et al.* (2014) “*High EphA2 Protein Expression In Renal Cell Carcinoma Is Associated With A Poor Disease Outcome*,” *Oncol. Lett.* Aug 2014; 8(2): 687-692; Miao, B. *et al.* (2014) “*EphA2 is a Mediator of Vemurafenib Resistance and a Novel Therapeutic Target in Melanoma*,” *Cancer Discov.* pii: CD-14-0295. EphA2 does not appear to be merely a marker for cancer, but rather appears to be persistently overexpressed and functionally changed in numerous human cancers (Chen, P. *et al.* (2014) “*Epha2 Enhances The Proliferation And Invasion Ability Of Lncap Prostate Cancer Cells*,” *Oncol. Lett.* 8(1):41-46).

[00135] The invention particularly contemplates the selection of EphA2 as a Cancer Antigen, and the use of anti-EphA2 antibodies to provide the Cancer Antigen-Binding Domain of the Tri-Specific Binding Molecules of the present invention. Exemplary anti-EphA2 antibodies include “**EphA2 mAb 1**,” “**EphA2 mAb 2**” and “**EphA2 mAb 3**.”

a. EphA2 mAb 1

[00136] The amino acid sequence of the VL Domain of a preferred anti-human EphA2 antibody ("EphA2 mAb 1") (SEQ ID NO:153) is shown below (CDR residues are shown underlined):

DIQMTQTTSS LSASLGDRIT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GYTLYTFGGG
 TKLEIK

CDR_L1 of EphA2 mAb 1 (SEQ ID NO:154): **RASQDISNYLN**

CDR_L2 of EphA2 mAb 1 (SEQ ID NO:155): **YTSRLHS**

CDR_L3 of EphA2 mAb 1 (SEQ ID NO:156): **QQGYTLYT**

[00137] The VL Domain of EphA2 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:157) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga
 cagaatcacc atcagttgca a gggcaagtca ggacattagc aattatttaa
actgggtatca gcagaaacca gatggaactg ttaaactcct gatctacttac
acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc
 tggaacagat tattctctca ccattagcaa cctggagcaa gaagatattg
 ccacttactt ttgccaacag ggttatacgc tgtacacgtt cggagggggg
 accaagctgg aaataaaa

[00138] The amino acid sequence of the VH Domain of EphA2 mAb 1 (SEQ ID NO:158) is shown below (CDR residues are shown underlined):

QVQLKESGPG LVAPSQSLSI TCTVSGFSLS RYSVHWVRQP PGKGLEWLGM
IWGGGSTDYN SALKSRLSIS KDNSKSQVFL KMNSLQTDDT AMYYCARKKHG
NYTMDYWGQ GTSVTVSS

CDR_H1 of EphA2 mAb 1 (SEQ ID NO:159): **GFSLSRYSVH**

CDR_H2 of EphA2 mAb 1 (SEQ ID NO:160): **MIWGGGSTDYNSALKS**

CDR_H3 of EphA2 mAb 1 (SEQ ID NO:161): **KHGNYYTMDY**

[00139] The VH Domain of EphA2 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:162) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

caggtgcagc tgaaggagtc aggacctggc ctggtggcac cctcacagag
 cctgtccatc acatgcactg tctctggggtt ctcattatcc agatatagtg
tacactgggt tcgccagcct ccaggaaagg gtctggagtg gctgggaatg

atatgggggtg gtggaagcac agactataat tcagctctca aatccagact
gagtatcagc aaggacaact ccaagagcca agttttctta aaaatgaaca
gtctgcaaac tgatgacaca gccatgtact actgtgccag aaaacatggt
aactactata ctatggacta ctgggggtcaa ggaacctcag tcaccgtctc
ctcc

b. EphA2 mAb 2

[00140] The amino acid sequence of the VL Domain of a second preferred anti-human EphA2 antibody (“EphA2 mAb 2”) (SEQ ID NO:163) is shown below (CDR residues are shown underlined):

DVVMQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
TFGSGTKLEI K

CDR_L1 of EphA2 mAb 2 (SEQ ID NO:164): **RSSQSLVHSSGNTYLH**

CDR_L2 of EphA2 mAb 2 (SEQ ID NO:165): **KVSNRFS**

CDR_L3 of EphA2 mAb 2 (SEQ ID NO:166): **SQSTHVP**

[00141] The VL Domain of EphA2 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:318) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gatgttggtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcttgca gatctagtca gagccttgta cacagtagtg
gaaacaccta tttacattgg tacctgcaga agccaggcca gtctccaaag
ctcctgatct acaaagtttc caaccgattt tctgggggtcc cagacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tatttctgtt ctcaaagtac acatgttccc
acgttcggct cggggacaaa gttggaaata aaa

[00142] The amino acid sequence of the VH Domain of EphA2 mAb 2 (SEQ ID NO:167) is shown below (CDR residues are shown underlined):

QIQLVQSGPE LKKPGETVKI SCKASGFTFT NYGMNWVKQA PGKGLKWMGW
INTYIGEPTY ADDFKGRFVF SLETSASTAY LQINNLIKNE D MATYFCAREEL
GPYYFDYWGQ GTTLTVSS

CDR_H1 of EphA2 mAb 2 (SEQ ID NO:168): **GFTFTNYGMN**

CDR_H2 of EphA2 mAb 2 (SEQ ID NO:169): **WINTYIGEPTYADDFKG**

CDR_H3 of EphA2 mAb 2 (SEQ ID NO:170): **ELGPYYFDY**

[00143] The VH Domain of EphA2 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:171) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

cagatccagt tgggtgcagtc tggacctgag ctgaagaagc ctggagagac
 agtcaagatc tcctgcaagg cttctgggtt taccttcaca aactatggaa
tgaactgggt gaagcaggct ccaggaaagg gtttaaagtg gatgggctgg
ataaacacct atattggaga gccgacatat gctgatgact tcaagggacg
 gtttgtcttc tctttgaaa cctctgccag cactgcctat ttgcagatca
 acaacctcaa aaatgaggac atggccacat atttctgtgc aagagaactg
ggaccatact actttgacta ctggggccaa ggcaccactc tcacagtctc
 ctcc

c. EphA2 mAb 3

[00144] The amino acid sequence of the VL Domain of a further preferred anti-human EphA2 antibody (“EphA2 mAb 3”) (SEQ ID NO:172) is shown below (CDR residues are shown underlined):

DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAVAWYQQKP GQSPKLLIFW
ASTRHAGVDP RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
 GTKLEIK

CDR_L1 of EphA2 mAb 3 (SEQ ID NO:173): **KASQDVTTAVA**

CDR_L2 of EphA2 mAb 3 (SEQ ID NO:174): **WASTRHA**

CDR_L3 of EphA2 mAb 3 (SEQ ID NO:175): **QQHYSTPYT**

[00145] The VL Domain of EphA2 mAb 3 is preferably encoded by a polynucleotide (SEQ ID NO:176) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gacattgtgc tgaccagtc tcacagatcc atgtccacat cagtaggaga
 caggggtcaac atcacctgca a aggccagtcaggatgtgact actgctgtag
cctgggtatca acaaaaacca gggcaatctc ctaaattact gatcttcttgg
gcatccaccc ggcacgctgg agtccctgat cgcttcacag gcagtggatc
 tgggacagat ttactctca ccatcagcag tgtgcaggct ggagacctgg
 cactttatta ctgtcaacaa cattatagca caccgtacac attcggaggg
 gggaccaagc tggaaataaa a

[00146] The amino acid sequence of the VH Domain of EphA2 mAb 3 (SEQ ID NO:177) is shown below (CDR residues are shown underlined):

EVQLVESGGG SVKPGGSLKL SCAASGFTFT DHYMYWVRQT PEKRLEWVAT
ISDGSFSTY PDSVKGRFTI SRDIAKNNLY LQMSSLKSED TAMYYCTRDE
SDRPFPYWGQ GTLVTVSS

CDR_{H1} of EphA2 mAb 3 (SEQ ID NO:178): **GFTFTDHYMY**

CDR_{H2} of EphA2 mAb 3 (SEQ ID NO:179): **TISDGGSFSTYPDSVKG**

CDR_{H3} of EphA2 mAb 3 (SEQ ID NO:180): **DESDRPFPY**

[00147] The V_H Domain of EphA2 mAb 3 is preferably encoded by a polynucleotide (SEQ ID NO:319) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

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gaagtgcagc tgggtggagtc tgggggaggc tcagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cactttcact gaccattaca
tgtatttgggt tcgccagact ccggaaaaga ggctggagtg ggtcgcaacc
attagtgatg gcggtagttt cacctcctat ccagacagtg tgaaggggcg
attcaccatc tccagagaca ttgccaagaa caacctgtac ctccaaatga
gcagtctgaa gtctgaggac acagccatgt attactgtac aagagatgag
agcgataggc cgtttcctta ctggggccaa gggactctgg tcactgtctc
ctcc
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6. gpA33-Binding Domains

[00148] gpA33 is also a preferred cancer antigen of the present invention. Colorectal cancer is among the most common malignancies of the Western world and is a leading cause of cancer deaths (Silverberg, E. *et al.* (1989) “*Cancer Statistics, 1989*,” CA Cancer J Clin. 39(1):3-20). One potentially useful target for colon cancer is the 43kD transmembrane glycoprotein A33 (**gpA33**), which is expressed in >95% of all colorectal carcinomas (Heath, J.K. *et al.* (1997) “*The Human A33 Antigen Is A Transmembrane Glycoprotein And A Novel Member Of The Immunoglobulin Superfamily*,” Proc. Natl. Acad. Sci. (U.S.A.) 94(2):469-474; Ritter, G. *et al.* (1997) “*Characterization Of Posttranslational Modifications Of Human A33 Antigen, A Novel Palmitoylated Surface Glycoprotein Of Human Gastrointestinal Epithelium*,” Biochem. Biophys. Res. Commun. 236(3):682-686; Wong, N.A. *et al.* (2006) “*EpCAM and gpA33 Are Markers Of Barrett's Metaplasia*,” J. Clin. Pathol. 59(3):260-263). gpA33 was first discovered through raising monoclonal murine antibodies against the human pancreatic carcinoma derived cell line ASPC1.

[00149] The invention particularly contemplates the selection of gpA33 as a Cancer Antigen, and the use of anti-gpA33 antibodies to provide the Cancer Antigen-Binding Domain of the Tri-Specific Binding Molecules of the present invention. An exemplary anti-gpA33 antibody is “**gpA33 mAb 1**.”

[00150] The amino acid sequence of the VL Domain of a preferred anti-human gpA33 antibody ("gpA33 mAb 1") (SEQ ID NO:181) is shown below (CDR residues are shown underlined):

DIQLTQSPSF LSASVGDRVIT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
 TKLEIK

CDR_L1 of gpA33 mAb 1 (SEQ ID NO:182): **SARSSISFMY**

CDR_L2 of gpA33 mAb 1 (SEQ ID NO:183): **DTSNLAS**

CDR_L3 of gpA33 mAb 1 (SEQ ID NO:184): **QQWSSYPLT**

[00151] The VL Domain of gpA33 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:185) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
 tcgagtgcatt attacttgct t ctgctaggctc ctcaatcagc ttcattgtact
 ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
 aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
 cctactattg cagcagtgagg agcagctatc ctctgacctt cggacagggg
 actaaactgg aaatcaag

[00152] The amino acid sequence of the VH Domain of gpA33 mAb 1 (SEQ ID NO:186) is shown below (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIIY
GNNVYFDVWG QGTTVTVSS

CDR_H1 of gpA33 mAb 1 (SEQ ID NO:187): **GYTFTGSWMN**

CDR_H2 of gpA33 mAb 1 (SEQ ID NO:188): **RIYPGDGETNYNGKFKD**

CDR_H3 of gpA33 mAb 1 (SEQ ID NO:189): **IYGNNVYFDV**

[00153] The VH Domain of gpA33 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:190) having the sequence shown below (polynucleotides encoding the CDRs are shown in underline):

caggtccagc tgggtccagag cggggccgaa gtcaaaaaaac ccggagcaag
 cgtgaagggtc tcctgcaaag catcaggcta tacatttaca ggcagctgga
tgaactgggt gaggcaggct ccaggacagg gactggagtg gatcgggcgc
atctaccctg gagacggcga aactaactat aatggaaagt tcaaagaccg

agtgaccatc acagccgata agtctactag taccgcctac atggagctga
 gctccctgcg gtctgaagat accgccgtct actattgcmc tagaatttac
ggaaacaatg tctattttga cgtgtggggg caggaacaa ctgtgactgt
 ctctctcc

7. Her2-Binding Domains

[00154] The invention also particularly contemplates the selection of Her2 as a Cancer Antigen, and the use of anti-Her2 antibodies to provide the Cancer Antigen-Binding Domain of the Tri-Specific Binding Molecules of the present invention. Exemplary anti-Her2 antibodies include “**Her2 mAb 1**” and **Trastuzumab**.

a. Her2 mAb 1

[00155] The amino acid sequence of the VL Domain of anti-Her2 antibody “**Her2 mAb 1**” (SEQ ID NO:191) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS
ASFLESGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
 GTKVEIKRT

[00156] The amino acid sequence of the VH Domain of anti-Her2 antibody “**Her2 mAb 1**” (SEQ ID NO:192) is shown below (CDR residues are shown underlined):

QVQLQQSGPE LVKPGASLKL SCTASGFNIK DTYIHWVKQR PEQGLEWIGR
IYPTNGYTRY DPKEQDKATI TADTSSNTAY LQVSRLTSED TAVYYCSRWG
GDGFYAMDYW GQGASVTVSS

b. Trastuzumab

[00157] The amino acid sequence of the VL Domain of the humanized anti-Her2 antibody “**Trastuzumab**” (SEQ ID NO:193) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS
ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
 GTKVEIKR

[00158] The amino acid sequence of the VH Domain of the humanized anti-Her2 antibody “**Trastuzumab**” (SEQ ID NO:194) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR
IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG
GDGFYAMDYW GQGTSLTVSS

8. B7-H3-Binding Domains

[00159] In addition to its expression on neuroblastoma cells, human B7-H3 is also known to be expressed on a variety of other cancer cells (e.g., gastric, ovarian and non-small cell lung cancers). B7-H3 protein expression has been immunohistologically detected in tumor cell lines (Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- γ Production*,” *Nature Immunol.* 2:269–274; Saatian, B. *et al.* (2004) “*Expression Of Genes For B7-H3 And Other T Cell Ligands By Nasal Epithelial Cells During Differentiation And Activation*,” *Amer. J. Physiol. Lung Cell. Mol. Physiol.* 287:L217–L225; Castriconi *et al.* (2004) “*Identification Of 4Ig-B7-H3 As A Neuroblastoma-Associated Molecule That Exerts A Protective Role From An NK Cell-Mediated Lysis*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 101(34):12640-12645); Sun, M. *et al.* (2002) “*Characterization of Mouse and Human B7-H3 Genes*,” *J. Immunol.* 168:6294-6297). mRNA expression has been found in heart, kidney, testes, lung, liver, pancreas, prostate, colon, and osteoblast cells (Collins, M. *et al.* (2005) “*The B7 Family Of Immune-Regulatory Ligands*,” *Genome Biol.* 6:223.1-223.7). At the protein level, B7-H3 is found in human liver, lung, bladder, testis, prostate, breast, placenta, and lymphoid organs (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 105(30):10277-10278).

[00160] The invention also particularly contemplates the selection of B7-H3 as a Cancer Antigen, and the use of anti-B7-H3 antibodies to provide the Cancer Antigen-Binding Domain of the Tri-Specific Binding Molecules of the present invention. Exemplary anti-B7-H3 antibodies include “**B7-H3 mAb 1**,” “**B7-H3 mAb 2**,” and “**B7-H3 mAb 3**.”

a. B7-H3 mAb 1

[00161] The amino acid sequence of the VL Domain of anti-B7-H3 antibody “**B7-H3 mAb 1**” (SEQ ID NO:195) is shown below (CDR residues are shown underlined):

DIAMTQSQKF MSTSVGDRVS VTCKASQNVD TNVAWYQQKP GQSPKALIYS
ASYRYSGVDP RFTGSGSGTD FTLTINNVSQ EDLAEYFCQQ YNNYPFTFGS
 GTKLEIK

[00162] The amino acid sequence of the VH Domain of anti-B7-H3 antibody “B7-H3 mAb 1” (SEQ ID NO:196) is shown below (CDR residues are shown underlined):

DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY
ISSDSSAIYY ADTVKGRFTI SRDNPKNLTF LQMTSLRSED TAMYICGRGR
ENIYYGSRLD YWGQGTTLTV SS

b. B7-H3 mAb 2

[00163] The amino acid sequence of the VL Domain of anti-B7-H3 antibody “B7-H3 mAb 2” (SEQ ID NO:197) is shown below (CDR residues are shown underlined):

DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQK DPQTVKLLIYY
TSRLHSGVPS RFGSGSGTD YSLTIDNLEQ EDIATYFCQQ GNTLPPTFGG
 GTKLEIK

[00164] The amino acid sequence of the VH Domain of anti-B7-H3 antibody “B7-H3 mAb 2” (SEQ ID NO:198) is shown below (CDR residues are shown underlined):

QVQLQQSGAE LARPGASVKL SCKASGYTFT SYWMQWVKQR PGQGLEWIGT
IYPGDGDTRY TQKFKGKATL TADKSSSTAY MQLSSLASED SAVYYCARRG
IPRLWYFDVW GAGTTVTSS

c. B7-H3 mAb 3

[00165] The amino acid sequence of the VL Domain of anti-B7-H3 antibody “B7-H3 mAb 3” (SEQ ID NO:199) is shown below (CDR residues are shown underlined):

DIQMTQSPAS LSVSVGETVT ITCRASESIY SYLAWYQQKQ GKSPQLLVYN
TKTLPEGVPS RFGSGSGTQ FSLKINSLOP EDFGRYYCQH HYGTPPWTFG
 GGTNLEIK

[00166] The amino acid sequence of the VH Domain of anti-B7-H3 antibody “B7-H3 mAb 3” (SEQ ID NO:200) is shown below (CDR residues are shown underlined):

EVQQVESGGD LVKPGGSLKL SCAASGFTFS SYGMSWVRQT PDKRLEWVAT
INSGGSNTYY PDSLKGRFTI SRDNAKNTLY LQMRSLKSED TAMYICARHD
GGAMDYWGQG TSVTVSS

9. EGF Receptor-Binding Domains (Cetuximab)

[00167] The amino acid sequence of the VL Domain of the chimeric anti-EGFR antibody “Cetuximab” (SEQ ID NO:201) is shown below (CDR residues are shown underlined):

DILLTQSPVI LSVSPGERVS FSCRASQSIG TNIHWYQQRT NGSPRLLIKY
ASESISGIPS RFSGSGSGTD FTLTINSVES EDIADYYCQQ NNNWPTTFGA
 GTKLELKR

[00168] The amino acid sequence of the VH Domain of the chimeric anti-EGFR antibody “Cetuximab” (SEQ ID NO:202) is shown below (CDR residues are shown underlined):

QVQLKQSGPG LVQPSQSLSI TCTVSGFSLT NYGVHWVRQS PGKGLEWLGV
IWSGNTDYN TPFTSRLSIN KDNSKSQVFF KMNSLQSDNT AIYYCARALT
YYDYEFAYWG QGTLVTVSA

[00169] Panitumumab (e.g., Vectibix®, Amgen) is an alternative EGF receptor-binding antibody that may be used in accordance with the present invention.

10. VEGF-Binding Domains (Bevacizumab)

[00170] The amino acid sequence of the VL Domain of the humanized anti-VEGF antibody “Bevacizumab” (SEQ ID NO:203) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIF
TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
 GTKVEIKR

[00171] The amino acid sequence of the VH Domain of the humanized anti-VEGF antibody “Bevacizumab” (SEQ ID NO:204) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTLLT VSS

11. 5T4-Binding Domains

[00172] The oncofetal protein, 5T4, is a tumor-associated protein displayed on the cell membrane of many carcinomas, including kidney, colon, prostate, lung, carcinoma and in acute lymphoblastic leukemia (see, Boghaert, E.R. *et al.* (2008) “The Oncofetal Protein, 5T4, Is A Suitable Target For Antibody-Guided Anti-Cancer

Chemotherapy With Calicheamicin,” Int. J. Oncol. 32(1):221-234; Eisen, T. *et al.* (2014) “*Naptumomab Estafenatox: Targeted Immunotherapy with a Novel Immunotoxin,*” Curr. Oncol. Rep. 16:370, pp. 1-6). The amino acid sequence of the Light Chain Variable Domain of an exemplary anti-5T4 antibody (“**5T4 mAb 1**”) is shown below (CDR residues are shown underlined): (SEQ ID NO:308):

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWWFQQKP GKAPKSLIYR
ANRLQSGVPS RFGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
 GTKLEIK

[00173] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary anti-5T4 antibody is shown below (CDR residues are shown underlined): (SEQ ID NO:309):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SFWMHWVRQA PGQGLEWMGR
IDPNRGGTEY NEKAKSRVTM TADKSTSTAY MELSSLRSED TAVYYCAGGN
PYYPMDYWGQ GTTVTVSS

[00174] The amino acid sequence of the Light Chain Variable Domain of a second exemplary anti-5T4 antibody (“**5T4 mAb 2**”) is shown below (CDR residues are shown underlined): (SEQ ID NO:310):

DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK
 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHPV
FTFGSGTKLE IK

[00175] The amino acid sequence of the Heavy Chain Variable Domain of such second exemplary anti-5T4 antibody is shown below (CDR residues are shown underlined) (SEQ ID NO:311):

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYWITWVKQR PGQGLEWIGD
IYPGSGRANY NEKFSKATL TVDTSSSTAY MQLSSLTSED SAVYNCARYG
PLFTTVVDPN SYAMDYWGQG TSVTVSS

12. IL13R α 2-Binding Domains

[00176] Interleukin-13 Receptor α 2 (**IL13R α 2**) is overexpressed in a variety of cancers, including glioblastoma, colorectal cancer, cervical cancer, pancreatic cancer, multiple melanoma, osteosarcoma, leukemia, lymphoma, prostate cancer and lung cancer (PCT Publication No. WO 2008/146911;

[00177] Brown, C.E. *et al.* (2013) “Glioma IL13R α 2 Is Associated With Mesenchymal Signature Gene Expression And Poor Patient Prognosis,” PLoS One.

18;8(10):e77769; Barderas, R. *et al.* (2012) “High Expression Of IL-13 Receptor A2 In Colorectal Cancer Is Associated With Invasion, Liver Metastasis, And Poor Prognosis,” *Cancer Res.* 72(11):2780-2790; Kasaian, M.T. *et al.* (2011) “IL-13 Antibodies Influence IL-13 Clearance In Humans By Modulating Scavenger Activity Of IL-13R α 2,” *J. Immunol.* 187(1):561-569; Bozinov, O. *et al.* (2010) “Decreasing Expression Of The Interleukin-13 Receptor IL-13R α 2 In Treated Recurrent Malignant Gliomas,” *Neurol. Med. Chir. (Tokyo)* 50(8):617-621; Fujisawa, T. *et al.* (2009) “A novel role of interleukin-13 receptor alpha2 in pancreatic cancer invasion and metastasis,” *Cancer Res.* 69(22):8678-8685). Antibodies that immunospecifically bind to IL13R α 2 are commercially available (Abnova Corporation, Biorbyt, LifeSpan BioSciences, United States Biologicals; see also PCT Publication No. WO 2008/146911). The amino acid sequence of the Light Chain Variable Domain of an exemplary anti-IL13R α 2 antibody (“**hu08**,” PCT Publication No. WO 2014/072888) is shown below (CDR residues are shown underlined): (SEQ ID NO:321):

DIQMTQSPSS LSASVGDRVT ITCKASQDVG TAVAWYQQKP GKAPKLLIYS
ASYRSTGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQH HYSAPWTFGG
 GTKVEIK

[00178] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary anti-IL13R α 2 antibody (“**hu08**,” PCT Publication No. WO 2014/072888) is shown below (CDR residues are shown underlined): (SEQ ID NO:322):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS RNGMSWVRQA PGKGLEWVAT
VSSGGSYIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARQG
TTALATRFFD VWGQGTLLTV SS

13. Integrin Beta6-Binding Domains

[00179] Integrin β 6 (**ITGB6**) is a subtype of integrin that is expressed exclusively on the surfaces of epithelial cells and is a receptor for extracellular matrix (ECM) proteins. ITGB6 expression is specifically expressed in tumor tissues (such as those of colon, prostate, kidney cancer), but is generally undetectable in healthy epithelial tissue (Liang, B. *et al.* (2014) “*Integrin β 6-targeted Immunoliposomes Mediate Tumor Specific Drug Delivery and Enhance Therapeutic Efficacy in Colon Carcinoma*,” *Clin. Cancer Res.* Dec 30. pii: clincanres.1194.2014). Monoclonal antibodies that immunospecifically bind to ITGB6 are available commercially (e.g., MAB2075Z clone R6G9, EMD Millipore; see also, Weinacker, A. *et al.* (1994) “*Role Of The*

Integrin Alpha V Beta 6 In Cell Attachment To Fibronectin. Heterologous Expression Of Intact And Secreted Forms Of The Receptor,” J. Biol. Chem. 269:6940-6948). Anti-ITGB6 monoclonal antibodies **3G9** and **8G6**, and variants thereof are disclosed in PCT Publication Nos. WO 03/100033 and WO 2007/008712.

[00180] The amino acid sequence of the Light Chain Variable Domain of an exemplary humanized anti-ITGB6 antibody (derived from antibody 3G9, PCT Publication No. WO 2007/008712) is shown below (CDR residues are shown underlined): (SEQ ID NO:312):

EIVLTQSPAT LSLSPGERAT LSC**SASSSVS SSYLY**WYQQK PGQAPRLLIY
STSNLASGIP ARFSGSGSGT GFTLTISSE PEDFAVYY**CH QWSTYPPT**FG
 GGTKVEIK

[00181] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary humanized anti-ITGB6 antibody (derived from antibody 3G9, PCT Publication No. WO 2007/008712) is shown below (CDR residues are shown underlined): (SEQ ID NO:313):

EVQLVESGGG LVQPGGSLRL SCAAS**GFTFS RYWMS**WVRQA PGKGLEWVAS
ISSGGRMYYP FTVKGRFTIS RDNAKNSLYL QMNSLRAEDT AVYYCARG**SI**
YDGYVFPYW GQGTTLTVSS

[00182] The amino acid sequence of the Light Chain Variable Domain of an exemplary anti-ITGB6 antibody (derived from antibody 8G6, PCT Publication No. WO 2007/008712) is shown below (CDR residues are shown underlined): (SEQ ID NO:314):

EIVLTQSPAT LSLSPGERAT LSC**RASQSVS TSSYSYMY**WY QQKPGQAPRL
 LIY**YASNLES** GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YC**QHNWEIPF**
TFGGGTKVEI K

[00183] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary anti-ITGB6 antibody (derived from antibody 8G6, PCT Publication No. WO 2007/008712) is shown below (CDR residues are shown underlined): (SEQ ID NO:315):

QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTDYAMH**WVRQAPGQGLEWMG**VISTYY**
GNTNYNQKFKGRVTMTRDTSISTAYMELSRSDDTAVYYCAR**GGLRRGDRPSLQ**
YAMDYWGQGTTLTVSS

14. Additional Anti-Cancer Antigen-Binding Domains

[00184] Additional anti-cancer antigen antibodies that may be used in accordance with the present invention include the following commercially available antibodies: **Brentuximab** (e.g., Adcetris®), which binds to CD30; **Gemtuzumab** (e.g., Mylotarg®, Wyeth), which binds to CD33; and **Ipilimumab** (e.g., Yervoy®), which binds to CTLA-4.

C. Exemplary Effector Cell-Binding Domains

[00185] Antibodies that are capable of binding to immune system effector cells may be used to provide the Effector Cell-Binding Domains of the Tri-Specific Binding Molecules of the present invention. Particularly suitable are antibodies that bind to CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, the B cell Receptor (BCR), the T cell Receptor (TCR), and the NKG2D Receptor.

1. CD2-Binding Domains

[00186] CD2 is a cell adhesion molecule found on the surface of T cells and natural killer (NK) cells. CD2 enhances NK cell cytotoxicity, possibly as a promoter of NK cell nanotube formation (Mace, E.M. *et al.* (2014) “*Cell Biological Steps And Checkpoints In Accessing NK Cell Cytotoxicity*,” Immunol. Cell. Biol. 92(3):245-255; Comerci, C.J. *et al.* (2012) “*CD2 Promotes Human Natural Killer Cell Membrane Nanotube Formation*,” PLoS One 7(10):e47664:1-12). The amino acid sequence of the VL Domain of anti-CD2 antibody (Lo-CD2a; ATCC Accession No: 11423) is (SEQ ID NO:102) (CDR residues are shown underlined):

DVVLTPPT LLATIGQSVS ISCRRSSQSL HSSGNTYLNW LLQRTGQSPQ
PLIYLVSKLE SGVPNRFSGS GSGTDFTLKI SGVEAEDLGV YYCMQFTHYP
YTFGAGTKLE LK

[00187] The amino acid sequence of the VH Domain of anti-CD2 antibody (Lo-CD2a; ATCC Accession No: 11423) is (SEQ ID NO:103) (CDR residues are shown underlined):

EVQLQQSGPE LQRPQASVKL SCKASGYIFT EYYMYWVKQR PKQGLELVGR
IDPEDGSIDY VEKFKKATL TADTSSNTAY MQLSSLTSED TATYFCARGK
FNYRFAYWGQ GTLVTVSS

2. CD3-Binding Domains

[00188] In a preferred embodiment, the second epitope that is bound by the Tri-Specific Binding Molecules of the present invention will be an epitope of CD3. CD3 is a T cell co-receptor composed of four distinct chains (Wucherpennig, K.W. *et al.* (2010) “*Structural Biology Of The T-Cell Receptor: Insights Into Receptor Assembly, Ligand Recognition, And Initiation Of Signaling*,” Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14). In mammals, the complex contains a CD3 γ chain, a CD3 δ chain, and two CD3 ϵ chains. These chains associate with a molecule known as the T cell receptor (TCR) in order to generate an activation signal in T lymphocytes. In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*,” Immunology 129(2):170–177). CD3 is found bound to the membranes of all mature T cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE,” 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) “*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The CD3 ϵ : γ Heterodimer*,” Cell 105(7):913-923; Kuhns, M.S. *et al.* (2006) “*Deconstructing The Form And Function Of The TCR/CD3 Complex*,” Immunity. 2006 Feb;24(2):133-139).

[00189] As discussed below, in order to illustrate the present invention, bi-specific anti-human CD3 x anti-human DR5-binding molecules were produced. An anti-human CD3 antibody used for such constructs is designated herein as “**CD3 mAb 2**.” The amino acid sequence of the VL Domain of **CD3 mAb 2 (SEQ ID NO:104)** is shown below (CDR residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TCR**RSSTGAVT** **TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWV**F
GGGTKLTVLG

CDR_L1 of CD3 mAb 2 (SEQ ID NO:105): **RSSTGAVTTSNYAN**

CDR_L2 of CD3 mAb 2 (SEQ ID NO:106): **GTNKRAP**

CDR_L3 of CD3 mAb 2 (SEQ ID NO:107): **ALWYSNLWV**

[00190] The amino acid sequence of the VH Domain of **CD3 mAb 2** (SEQ ID NO:108) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS

CDR_H1 of CD3 mAb 2 (SEQ ID NO:109): **TYAMN**

CDR_H2 of CD3 mAb 2 (SEQ ID NO:110): **RIRSKYNNYATYYADSVKD**

CDR_H3 of CD3 mAb 2 (SEQ ID NO:111): **HGNFGNSYVSWFAY**

[00191] In some of the CD3 constructs, a variant VH Domain was employed for **CD3 mAb 2**. The variant VH domain possesses a D65G substitution, thus having the amino acid sequence shown below (SEQ ID NO:112) (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKGDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS

[00192] The substitution causes the CDR_H2 to have the amino acid sequence (SEQ ID NO:113) RIRSKYNNYATYYADSVKG. The substituted position (D65G) is shown in double underline.

[00193] A second anti-CD3 antibody used herein is antibody Muromonab-CD3 “**OKT3**” (Xu *et al.* (2000) “*In Vitro Characterization Of Five Humanized OKT3 Effector Function Variant Antibodies*,” Cell. Immunol. 200:16-26); Norman, D.J. (1995) “*Mechanisms Of Action And Overview Of OKT3*,” Ther. Drug Monit. 17(6):615-620; Canafax, D.M. *et al.* (1987) “*Monoclonal Antilymphocyte Antibody (OKT3) Treatment Of Acute Renal Allograft Rejection*,” Pharmacotherapy 7(4):121-124; Swinnen, L.J. *et al.* (1993) “*OKT3 Monoclonal Antibodies Induce Interleukin-6 And Interleukin-10: A Possible Cause Of Lymphoproliferative Disorders Associated With Transplantation*,” Curr. Opin. Nephrol. Hypertens. 2(4):670-678). The amino acid sequence of the VL Domain of **OKT3** (SEQ ID NO:114) is shown below (CDR residues are shown underlined):

QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT
SKLASGVPAAH FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG
 TKLEINR

[00194] The amino acid sequence of the VH Domain of **OKT3 (SEQ ID NO:115)** is shown below (CDR residues are shown underlined):

QVQLQQSGAE LARPGASVKM SCKASGYTFT **RYTMH**WVKQR PGQGLEWIGY
INPSRGY**TNY** **NQKFKD**KATL TTDKSSSTAY MQLSSLTSED SAVYYCAR**YY**
DDHYCLDYWG QGTTLTVSS

3. CD16-Binding Domains

[00195] CD16 is the FcγRIIIA receptor. CD16 is expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that bind aggregated but not monomeric human IgG (Peltz, G.A. *et al.* (1989) “*Human Fc Gamma RIII: Cloning, Expression, And Identification Of The Chromosomal Locus Of Two Fc Receptors For IgG*,” Proc. Natl. Acad. Sci. (U.S.A.) 86(3):1013-1017; Bachanova, V. *et al.* (2014) “*NK Cells In Therapy Of Cancer*,” Crit. Rev. Oncog. 19(1-2):133-141; Miller, J.S. (2013) “*Therapeutic Applications: Natural Killer Cells In The Clinic*,” Hematology Am. Soc. Hematol. Educ. Program. 2013:247-253; Youinou, P. *et al.* (2002) “*Pathogenic Effects Of Anti-Fc Gamma Receptor IIIB (CD16) On Polymorphonuclear Neutrophils In Non-Organ-Specific Autoimmune Diseases*,” Autoimmun Rev. 1(1-2):13-19; Peipp, M. *et al.* (2002) “*Bi-specific Antibodies Targeting Cancer Cells*,” Biochem. Soc. Trans. 30(4):507-511).

[00196] The amino acid sequence of a Variable Light Chain Domain of anti-CD16 antibody 3G8 is (**SEQ ID NO:116**) (CDR residues are shown underlined):

DTVLTQSPAS LAVSLGQRAT ISC**KASQSVD** **FDGDSFMN**WY QQKPGQPPKL
 LIY**TTSNLES** GIPARFSASG SGTDFTLNIH PVEEEDTATY YC**QQS****NEDPY**
TFGGGTKLEI K

[00197] The amino acid sequence of the Variable Heavy Chain Domain of anti-CD16 antibody 3G8 is (**SEQ ID NO:117**) (CDR residues are shown underlined):

QVTLKESGPG ILQPSQTLSL TCSFSGFSLR **TSGMGV**GWIR QPSGKGLEWL
 A**HIW****DDDKR** **YNPALKS**RLT ISKDTSSNQV FLKIASVDTA DTATYYCAQ**I**
NPAWFAYWGQ GTLVTVSA

[00198] The amino acid sequence of a Variable Light Chain Domain of anti-CD16 antibody A9 is (**SEQ ID NO:118**) (CDR residues are shown underlined):

DIQAVVTQES ALTTSPPGETV TLTC**RSNTGT** **VTTSNYAN**WV QEKPDHLEFG
 LIG**HTNNRAP** GVPARFSGSL IGDKAALTIT GAQTEDEAIY FC**ALWYNNHW**
VFGGGTKLTVL

[00199] The amino acid sequence of the Variable Heavy Chain Domain of anti-CD16 antibody A9 is (SEQ ID NO:119) (CDR residues are shown underlined):

QVQLQQSGAE LVRPGTSVKI SCKASGYTFT NYWLGWVKQR PGHGLEWIGD
IYPGGGYTNY NEKFKGKATV TADTSSRTAY VQVRSLTSED SAVYFCARSA
SWYFDVWGAR TTVTVSS

4. CD19-Binding Domains

[00200] CD19 antigen is a type I transmembrane glycoprotein belonging to the immunoglobulin Ig superfamily. CD19 is expressed on follicular dendritic cells and B cells. It is considered a pan B cell marker expressed throughout B cell development but with threefold higher expression in mature cells as compared to immature B cells (Raufi A. *et al.* (2013) “*Targeting CD19 In B-Cell Lymphoma: Emerging Role Of SAR3419*,” Cancer Manag. Res. 5:225-233). Many CD19 antibodies have been described (*e.g.*, MD1342, MEDI-551, *etc.*) (Mei, H.E. *et al.* (2012) “*Rationale Of Anti-CD19 Immunotherapy: An Option To Target Autoreactive Plasma Cells In Autoimmunity*,” Arthritis Res. Ther. 14(Suppl 5):S1:1-16). The anti-CD19 binding molecule “blinatumomab” is disclosed in EP 2186527.

[00201] The amino acid sequence of the VL Domain of a preferred anti-CD19 antibody (HD37) is (SEQ ID NO:120) (CDR residues are shown underlined):

DILITQSPKS MSMSVGERVT LTCKASENVV TYVSWYQQKP EQSPKLLIYG
ASNRYTGVDP RFTGSGSATD FTLTISSVQA EDLADYHCGQ GYSYPYTFGG
 GTKLEIKR

[00202] The amino acid sequence of the VH Domain of anti-CD19 antibody HD37 is (SEQ ID NO:121) (CDR residues are shown underlined):

QVQLQQSGAE LVRPGSSVKI SCKASGYAFS SYWMNWVKQR PGQGLEWIGQ
IWPGDGDTNY NGKFKGKATL TADESSSTAY MQLSSLASED SAVYFCARRE
TTTVGRYYA MDYWGQTSV TVSS

5. CD20-Binding Domains

[00203] CD20 is a B cell-specific differentiation antigen that is expressed on mature B cells and in most B cell non-Hodgkin's lymphomas but not on early B cell progenitors or later mature plasma cells (Maloney, D.G. (2012) “*Anti-CD20 Antibody Therapy for B-Cell Lymphomas*,” N. Engl. J. Med. 366:2008-2016). Rituximab is an illustrative anti-human CD20 antibody. The amino acid sequence of the VL Domain

of a chimeric anti-CD20 antibody (rituximab) is (SEQ ID NO:122) (CDR residues are shown underlined):

QIVLSQSPAI LSASPGKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT
SNLASGVPVR FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG
 TKLEIKR

[00204] The amino acid sequence of the VH Domain of anti-CD20 antibody (rituximab) is (SEQ ID NO:123) (CDR residues are shown underlined):

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA
IYPNGDTSY NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST
YYGGDWYFNV WGAGTTTVTS A

[00205] Alternative anti-CD20 antibodies that may be used in accordance with the present invention include the following commercially available antibodies: **Ibritumomab** (e.g., Zevalin®, Spectrum Pharmaceuticals, Inc.), **Ofatumumab** (e.g., Arzerra®, SmithKlineGlaxo) and **Tositumomab** (e.g., Bexxar®, GlaxoSmithKline).

6. CD22-Binding Domains

[00206] CD22 is a sugar binding transmembrane protein found on the surface of mature B cells and to a lesser extent on some immature B cells (WO 2011/032633; Poe, J.C. *et al.* (2012) “CD22 And Siglec-G In B Cell Function And Tolerance,” Trends Immunol. 33(8):413-420; Chen, W.C. *et al.* (2012) “Targeting B Lymphoma With Nanoparticles Bearing Glycan Ligands Of CD22,” Leuk. Lymphoma 53(2):208-210; Walker, J.A. (2008) “CD22: An Inhibitory Enigma,” Immunology 123(3):314-325; Coleman, M. *et al.* (2003) “Epratuzumab: Targeting B-Cell Malignancies Through CD22,” Clin. Cancer Res. 9(10 Pt 2):3991S-3994S).

[00207] The amino acid sequence of the VL Domain of anti-CD22 antibody (epratuzumab) is (SEQ ID NO:124) (CDR residues are shown underlined):

DIQLTQSPSS LSASVGDRVT MSCKSSQSVL YSANHKNYLA WYQQKPGKAP
 KLLIYWASTR ESGVPSRFSG SGSGTDFTFIT ISSLPEDIA TYYCHQYLSS
WTFGGGTVKQ IKR

[00208] The amino acid sequence of the VH Domain of anti-CD22 antibody (epratuzumab) is (SEQ ID NO:125) (CDR residues are shown underlined):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYWLHWVRQA PGQGLEWIGY
INPRNDYTEY NQNFKDKATI TADESTNTAY MELSSLRSED TAFYFCARRD
ITTFYWGQGT TTVVSS

7. CD32B-Binding Domains

[00209] A preferred sequence for the VL domain of an antibody that binds to human CD32B is CD32B mAb 1 (SEQ ID NO:126) (CDR residues are shown underlined):

DIQMTQSPSS LLAALGERVS LTCRASQEIS GYLSWLQQKP DGTIKRLIYA
ASTLDSGVPK RFSGSESGSD YSLTISSLES EDFADYYCLQ YFSYPLTFGA
 GTKLELK

[00210] A preferred sequence for the VH domain of the CD32B mAb 1 antibody that binds to human CD32B is (SEQ ID NO:127) (CDR residues are shown underlined):

EVKLEESGGG LVQPGGSMKL SCEASGFTFS DAWMDWVRQS PEKGLEWVAE
IRNKAKNHAT YYAESVIGRF TISRDDSKSS VYLQMNSLRA EDTGIYYCGA
LGLDYWGQGT TLTVSS

8. CD64-Binding Domains

[00211] CD64 is the FcγRI receptor and is expressed on monocytes/macrophages, dendritic cells, and activated granulocytes. The expression can be upregulated by IFN-γ stimulation. CD64 binds IgG immune complex. CD64 plays a role in antigen capture, phagocytosis of IgG/antigen complexes, and antibody-dependent cellular cytotoxicity (WO 2006/002438).

[00212] A preferred sequence for the VL domain of an antibody that binds to human CD64 is CD64 mAb 1 (SEQ ID NO:128) (CDR residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASSRATGIPA RFGGSGSGGT DFTLTISSE PEDFAVYYCQ LRSNWPPYTF
 GQGTKLEIK

[00213] A preferred sequence for the VH domain of an antibody that binds to human CD64 is (SEQ ID NO:129) (CDR residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCAASGFIFS GYGMHWVRQA PGKGLEWVTV
IWYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDT
GDRFFDYWGQ GTLVTVSS

9. BCR/CD79-Binding Domains

[00214] The BCR is composed of a membrane immunoglobulin which, together with non-covalently associated α and β subunits of CD79 ("CD79a" and "CD79b,"

respectively), forms the BCR complex. CD79a and CD79b are signal transducing subunits that contain a conserved immunoreceptor tyrosine-based activation motif ("ITAM") required for signal transduction (Dylke, J. *et al.* (2007) "Role Of The Extracellular And Transmembrane Domain Of Ig-Alpha/Beta In Assembly Of The B Cell Antigen Receptor (BCR)," Immunol. Lett. 112(1):47-57; Cambier, J.C. (1995) "New Nomenclature For The Reth Motif (or ARH1/TAM/ARAM/YXXL)," Immunol. Today 16:110). Aggregation of the BCR complex by multivalent antigen initiates transphosphorylation of the CD79a and CD79b ITAMs and activation of receptor-associated kinases (DeFranco, A.L. (1997) "The Complexity Of Signaling Pathways Activated By The BCR," Curr. Opin. Immunol. 9:296-308; Kurosaki, T. (1997) "Molecular Mechanisms In B Cell Antigen Receptor Signaling," Curr. Opin. Immunol. 9:309-318; Kim, K.M. *et al.* (1993) "Signalling Function Of The B-Cell Antigen Receptors," Immun. Rev. 132:125-146). Phosphorylated ITAMs recruit additional effectors such as PI₃K, PLC- γ and members of the Ras/MAPK pathway. These signaling events are responsible for both the B cell proliferation and increased expression of activation markers (such as MHCII and CD86) that are required to prime B cells for their subsequent interactions with T helper ("T_h") cells.

[00215] A preferred sequence for the VL domain of an antibody that binds to the human B Cell Receptor (CD79) is CD79 mAb 1 (**SEQ ID NO:130**) (CDR residues are shown underlined):

DVVMQTPLT LSVNIGQPAS ISCKKSSQSLL DTDGKTYLNW LLQRPQGSPN
RLIYLVSKLD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGI YYCWQGTHFP
LTFGAGTKLE LK

[00216] A preferred sequence for the VH domain of the CD79 mAb 1 antibody that binds to the human B Cell Receptor (CD79) is (**SEQ ID NO:131**) (CDR residues are shown underlined):

QVQLQQPGAE LVRPGASVKL SCKASGYTFT SYWMNWVKQR PGQGLEWIGM
VDPSDSETHY NQMFKDKATL TVDKSSSTAY MQLSSLTSED SAVYYCARAM
GYWGQGTSTV VSS

10. T Cell Receptor-Binding Domains

[00217] In an alternate embodiment, the second epitope that is bound by the Tri-Specific Binding Molecules of the present invention will be an epitope of the T cell

Receptor (TCR). The T cell Receptor is natively expressed by CD4⁺ or CD8⁺ T cells, and permits such cells to recognize antigenic peptides that are bound and presented by class I or class II MHC proteins of antigen presenting cells. Recognition of a pMHC (peptide–MHC) complex by a TCR initiates the propagation of a cellular immune response that leads to the production of cytokines and the lysis of the antigen presenting cell (see, *e.g.*, Armstrong, K.M. *et al.* (2008) “*Conformational Changes And Flexibility In T-Cell Receptor Recognition Of Peptide–MHC Complexes*,” *Biochem. J.* 415(Pt 2):183–196; Willemsen, R. (2008) “*Selection Of Human Antibody Fragments Directed Against Tumor T-Cell Epitopes For Adoptive T-Cell Therapy*,” *Cytometry A.* 73(11):1093-1099; Beier, K.C. *et al.* (2007) “*Master Switches Of T-Cell Activation And Differentiation*,” *Eur. Respir. J.* 29:804-812; Mallone, R. *et al.* (2005) “*Targeting T Lymphocytes For Immune Monitoring And Intervention In Autoimmune Diabetes*,” *Am. J. Ther.* 12(6):534–550). CD3 is the receptor that binds to the TCR (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*,” *Immunology* 129(2):170-177; Guy, C.S. *et al.* (2009) “*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex*,” *Immunol. Rev.* 232(1):7-21; St. Clair, E.W. (Epub 2009 Oct 12) “*Novel Targeted Therapies For Autoimmunity*,” *Curr. Opin. Immunol.* 21(6):648-657; Baeuerle, P.A. *et al.* (Epub 2009 Jun 9) “*Bi-specific T-Cell Engaging Antibodies For Cancer Therapy*,” *Cancer Res.* 69(12):4941-4944; Smith-Garvin, J.E. *et al.* (2009) “*T Cell Activation*,” *Annu. Rev. Immunol.* 27:591-619; Renders, L. *et al.* (2003) “*Engineered CD3 Antibodies For Immunosuppression*,” *Clin. Exp. Immunol.* 133(3):307-309).

[00218] Antibodies that specifically bind to the T cell Receptor include the anti-TCR antibody BMA 031 (EP 0403156; Kurrle, R. *et al.* (1989) “*BMA 031 – A TCR-Specific Monoclonal Antibody For Clinical Application*,” *Transplant Proc.* 21(1 Pt 1):1017-1019; Nashan, B. *et al.* (1987) “*Fine Specificity Of A Panel Of Antibodies Against The TCR/CD3 Complex*,” *Transplant Proc.* 19(5):4270-4272; Shearman, C.W. *et al.* (1991) “*Construction, Expression, And Biologic Activity Of Murine/Human Chimeric Antibodies With Specificity For The Human α/β T Cell*,” *J. Immunol.* 146(3):928-935; Shearman, C.W. *et al.* (1991) “*Construction, Expression And Characterization of Humanized Antibodies Directed Against The Human α/β T Cell Receptor*,” *J. Immunol.* 147(12):4366-4373).

[00219] The amino acid sequence of the VL Domain of anti-TCR antibody BMA 031 is (SEQ ID NO:132) (CDR residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCSATSSVS YMHWYQQKPG KAPKRWIYDT
SKLASGVPSR FSGSGSGTEF TLTISSLQPE DFATYYCQQW SSNPLTFGQG
 TKLEIK

[00220] The amino acid sequence of a VH Domain of anti-TCR antibody BMA 031 is (SEQ ID NO:133) (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYKFT SYVMHWVRQA PGQGLEWIGY
INPYNDVTKY NEKFKGRVTI TADKSTSTAY LQMNSLRSED TAVHYCARGS
YYDYDGFVYW GQGTTLVTVSS

11. NKG2D Receptor-Binding Domains

[00221] In an alternate embodiment, the second epitope that is bound by the Tri-Specific Binding Molecules of the present invention will be an epitope of the NKG2D receptor. The NKG2D receptor is expressed on all human (and other mammalian) Natural Killer cells (Bauer, S. *et al.* (1999) "Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA," *Science* 285(5428):727-729; Jamieson, A.M. *et al.* (2002) "The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing," *Immunity* 17(1):19-29) as well as on all CD8⁺ T cells (Groh, V. *et al.* (2001) "Costimulation Of CD8 $\alpha\beta$ T Cells By NKG2D Via Engagement By MIC Induced On Virus-Infected Cells," *Nat. Immunol.* 2(3):255-260; Jamieson, A.M. *et al.* (2002) "The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing," *Immunity* 17(1):19-29). Such binding ligands, and particularly those which are not expressed on normal cells, include the histocompatibility 60 (H60) molecule, the product of the retinoic acid early inducible gene-1 (RAE-1), and the murine UL16-binding proteinlike transcript 1 (MULT1) (Raulet D.H. (2003) "Roles Of The NKG2D Immunoreceptor And Its Ligands," *Nature Rev. Immunol.* 3:781-790; Coudert, J.D. *et al.* (2005) "Altered NKG2D Function In NK Cells Induced By Chronic Exposure To Altered NKG2D Ligand-Expressing Tumor Cells," *Blood* 106:1711-1717). Antibodies that specifically bind to the NKG2D Receptor include KYK-2.0 (Kwong, KY *et al.* (2008) "Generation, Affinity Maturation, And Characterization Of A Human Anti-Human NKG2D Monoclonal Antibody With Dual Antagonistic And Agonistic Activity," *J. Mol. Biol.* 384:1143-1156; and PCT/US09/54911).

[00222] The amino acid sequence of the VL Domain of anti-NKG2D antibody KYK-1.0 is (SEQ ID NO:134) (CDR residues are shown underlined):

QPVLTPSSV SVAPGETARI PCGGDDIETK SVHWYQQKPG QAPVLVIYDD
DDRPSGIPER FFGSNSGNTA TLSISRVEAG DEADYYCQVW DDNNDEWVFG
 GGTQLTVL

[00223] The amino acid sequence of the VH Domain of anti-NKG2D antibody KYK-1.0 is (SEQ ID NO:135) (CDR residues are shown underlined):

EVQLVESGGG VVQPGGSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAF
IRYDGSNKYY ADSVKGRFTI SRDNSKNTKY LQMNSLRAED TAVYYCAKDR
FGYYLDYWGQ GTLVTVSS

[00224] The amino acid sequence of a VL Domain of anti-NKG2D antibody KYK-2.0 is (SEQ ID NO:136) (CDR residues are shown underlined):

QSALTQPASV SGSPGQSITI SCSGSSSNIG NNAVNWYQQL PGKAPKLLIY
YDDLPSGVs DRFSGSKSGT SAFLAISGLQ SEDEADYYCA AWDDSLNGPV
 FGGGTKLTVL

[00225] The amino acid sequence of a VH Domain of anti-NKG2D antibody KYK-2.0 is (SEQ ID NO:137) (CDR residues are shown underlined):

QVQLVESGGG LVKPGGSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAF
IRYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR
GLGDGTYFDY WGQGTTVTVS S

D. Preferred Trispecific Binding Molecules of the Present Invention

1. Preferred Fc Domains

[00226] The CH2 and CH3 Domains of the two heavy chains interact to form the **Fc Domain**, which is a domain that is recognized by cellular **Fc Receptors (FcγRs)**. As used herein, the term “Fc Domain” is used to define a C-terminal region of an IgG heavy chain. The amino acid sequence of the CH2-CH3 domain of an exemplary human IgG1 is (SEQ ID NO:1):

| CH2 →

APELLGGPS	VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT
231	240	250	260	270	280

←CH2 | CH3→

KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	K GQPREPQVY
290	300	310	320	330	340

TLPPSREEMT	KNQVSLTCLV	KGFYPSTIAV	EWESNGQPEN	NYKTTTPVLD	SDGSFFLYSK
350	360	370	380	390	400

←CH3 |

LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK	SLSLSPGK
410	420	430	440

[00227] Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by references. The “EU index as in Kabat” refers to the numbering of the human IgG1 EU antibody. Amino acids from the variable regions of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. Kabat’s numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[00228] Although boundaries may vary slightly, the CH2 domain of a human IgG Fc Domain usually extends from amino acids 231 to amino acid 341 of a human IgG according to the numbering system of Kabat. The CH3 domain of a human IgG usually extends from amino acids 342 to 447 according to the numbering system of Kabat. The “hinge region” or “hinge domain” is generally defined as stretching from Glu216 to Pro230 of human IgG1.

[00229] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the antibodies of the present invention may be incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein.

[00230] Activating and inhibitory signals are transduced through the Fc Receptors (FcγRs) following their ligation to an Fc Domain. These diametrically opposing functions result from structural differences among the different receptor isoforms. Two distinct domains within the cytoplasmic signaling domains of the receptor called immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs) account for the different responses. The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγR complexes include FcγRI, FcγRIIA, FcγRIIIA, whereas ITIM-containing complexes only include FcγRIIB. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs along with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, activation of which results in activation of downstream substrates (*e.g.*, PI₃K). Cellular activation leads to release of proinflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently

the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR-mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca⁺⁺. Thus cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness. B cell activation, B cell proliferation and antibody secretion is thus aborted.

[00231] The Fc Domain of the binding molecules of the present invention may be either a complete Fc Domain (*e.g.*, a complete IgG Fc Domain) or only a fragment of a complete Fc Domain. Although the Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such Fc Domain will cause altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Domain) or will substantially eliminate the ability of such Fc Domain to bind to inhibitory receptor(s). Thus, the Fc Domain of the Fc Domain-containing diabodies of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Domain, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Domain). Such Fc Domains may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Domains, or may comprise non-naturally occurring orientations of CH2 and/or CH3 domains (such as, for example, two CH2 domains or two CH3 Domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, etc.).

[00232] Fc Domain modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion*

In Vivo Via Low-Affinity Activating Fcγ Receptors,” Cancer Res. 57(18):8882-8890).

[00233] In particular, it is preferred for the CH2-CH3 domains of the polypeptide chains of the Fc Domain-containing diabodies of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (**SEQ ID NO:1**)). Fc variants and mutant forms capable of mediating such altered binding are described above. In a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such diabodies include any 1, 2, 3, 4, 5, 6, or 7 of the substitutions: L234A, L235A, F243L, R292P, Y300L, V305I and P396L. Exemplary variants of human IgG1 Fc Domains with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R292P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc Domain in any combination. In one embodiment, the human IgG1 Fc Domain variant contains a F243L, R292P and Y300L substitution. In another embodiment, the human IgG1 Fc Domain variant contains a F243L, R292P, Y300L, V305I and P296L substitution. In one embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such diabodies include any 1, 2, or 3, of the substitutions: L234A, L235A, N297G, N297Q. In another embodiment, the human IgG1 Fc Domain variant contains an N297Q substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 domain which inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:1**)) is utilized. In a specific embodiment, the Fc Domain-containing diabodies of the present invention comprise an IgG2 Fc Domain or an IgG4 Fc Domain. Where an IgG4 Fc Domain is utilized the instant invention also encompasses the introduction of a stabilizing mutation such as S228P, as numbered by the EU index as set forth in Kabat (Lu *et al.*, (2008) “*The Effect Of A Point Mutation On The Stability Of IgG4 As Monitored By Analytical Ultracentrifugation*,” J Pharmaceutical Sciences 97:960-969) to reduce the incidence of strand exchange. Other stabilizing mutations known in the art may be introduced into an IgG4 Fc Domain (Peters, P *et al.*, (2012)

“Engineering an Improved IgG4 Molecule with Reduced Disulfide Bond Heterogeneity and Increased Fab Domain Thermal Stability,” J. Biol. Chem., 287:24525-24533; PCT Patent Publication No: WO 2008/145142). Since the N297A, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00234] The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a “knob”, e.g., tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, i.e., “the hole” (e.g., a substitution with glycine). Such sets of mutations can be engineered into any two of the polypeptides of the Tri-Specific Binding Molecule. Methods of protein engineering to favor heterodimerization over homodimerization are well-known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see e.g., Ridgway *et al.* (1996) “‘Knobs-Into-Holes’ Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,” Protein Engr. 9:617-621, Atwell *et al.* (1997) “Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,” J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) “A New Format Of Bi-specific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,” J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety). Preferably the “knob” is engineered into the CH2-CH3 Domains of the first polypeptide chain and the “hole” is engineered into the CH2-CH3 Domains of the other CH2-CH3-containing polypeptide chain. Thus, the “knob” will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 Domains. The CH2-CH3 “hole-bearing” polypeptide chain will heterodimerize with the CH2-CH3 “knob-bearing” polypeptide chain, and will also homodimerize with itself. A preferred knob is created by modifying a native IgG Fc

Domain to contain the modification T366W. A preferred hole is created by modifying a native IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying the “hole-bearing” polypeptide chain homodimer from the final Tri-Specific Binding Molecule, the protein A binding site of the CH2 and CH3 Domains of the “hole-bearing” Fc Domain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the “hole-bearing” Fc Domain homodimer will not bind to protein A, whereas the desired Tri-Specific Binding Molecule will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[00235] A preferred sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Domain-containing diabody of the present invention will have the “knob-bearing” sequence (SEQ ID NO:52):

```
APEAAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME
ALHNHYTQKS LSLSPGK
```

[00236] A preferred sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Domain-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Domain-containing diabody having three polypeptide chains) will have the “hole-bearing” sequence (SEQ ID NO:53):

```
APEAAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVME
ALHNRYTQKS LSLSPGK
```

[00237] As will be noted, the CH2-CH3 Domains of SEQ ID NO:52 and SEQ ID NO:53 include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (SEQ ID NO:1)).

[00238] It is preferred that the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of **SEQ ID NO:52**. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:53**) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:52**) would be employed in the second polypeptide chain of an Fc Domain-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Domain-containing diabody having three polypeptide chains).

2. Preferred First Polypeptide Chain

[00239] A first polypeptide chain of a preferred binding molecule of the present invention will comprise a Variable Light Chain Domain capable of binding to Epitope I (**VL_I**), a Variable Heavy Chain Domain capable of binding to Epitope II (**VH_{II}**), a Heterodimer-Promoting Domain and a CH2-CH3 Domain.

[00240] Since the Variable Light Chain and Variable Heavy Chain Domains of the first polypeptide are directed toward different epitopes, they cannot associate together to form a Binding Domain that is able to bind either Epitope I or Epitope II. The Variable Light Chain and Variable Heavy Chain Domains of the first polypeptide are spaced apart from one another by an intervening linker peptide that is sufficiently short as to substantially prevent the association of these Domains. An exemplary linker, termed “**Linker 1**,” has the sequence (**SEQ ID NO:33**): GGGSGGGG.

[00241] The Variable Heavy Chain Domain of the first polypeptide and the Heterodimer-Promoting Domain of that polypeptide are preferably spaced apart from one another by an intervening linker peptide that contains 1, 2, 3 or more cysteine residues. A preferred cysteine-containing spacer peptide (“**Linker 2**”) has the sequence is **SEQ ID NO:34**: GGCGGG.

[00242] Linkers that may be employed to link a CH2-CH3 Domain to a polypeptide chain of the molecules of the present invention include: ASTKG (**SEQ ID NO:47**), DKTHTCPPCP (**SEQ ID NO:48**), LEPKSS (**SEQ ID NO:49**), and APSSSPME (**SEQ ID NO:50**), APSSS (**SEQ ID NO:152**) and GGG or GCG. **SEQ**

ID NO:49 may be used in lieu of GGG or GCG for ease of cloning. Additionally, **SEQ ID NO:49** may be immediately followed by **SEQ ID NO:47** to form an alternate linker (LEPKSSDKTHTCPPCP; **SEQ ID NO:51**).

[00243] The Heterodimer-Promoting Domain of the first polypeptide and the Heterodimer-Promoting Domain of the second polypeptide are coordinately selected. The Domains differ from one another and are designed to associate with one another so as to promote the association of the first and second polypeptide chains. For example, one of the Heterodimer-Promoting Domains will be engineered to have a negative charge at pH 7, while the other of the two polypeptide chains will be engineered to have a positive charge at pH 7. The presence of such charged Domains promotes association between the first and second polypeptides, and thus fosters heterodimerization. It is immaterial which Heterodimer-Promoting Domains is provided to which chain, as long as the Domains employed on the first and second polypeptide chains differ so as to foster heterodimerization between such chains.

[00244] The Heterodimer-Promoting Domains may be the IgG CL and CH1 domains or may be a peptide having the amino acid sequence GVEPKSC (**SEQ ID NO:35**) or VEPKSC (**SEQ ID NO:36**), derived from the hinge domain of a human IgG, and in lieu of the CL domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (**SEQ ID NO:37**) or FNRGEC (**SEQ ID NO:38**).

[00245] More preferably, however, the Heterodimer-Promoting Domains of such diabodies are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) “*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*,” *Biomacromolecules* 9:3173–3180; Arndt, K.M. *et al.* (2001) “*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*,” *J. Molec. Biol.* 312:221-228; Arndt, K.M. *et al.* (2002) “*Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils*,” *Structure* 10:1235-1248; Boucher, C. *et al.* (2010) “*Protein Detection By Western Blot Via Coiled–Coil Interactions*,”

Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) “*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*,” J. Mol. Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) “*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*,” Biochemistry 42:1754-1763; Fernandez-Rodriguez, J. *et al.* (2012) “*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*,” Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) “*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*,” Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) “*Structural Specificity In Coiled-Coil Interactions*,” Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002) “*Designing Heterodimeric Two-Stranded α -Helical Coiled-Coils: The Effects Of Hydrophobicity And α -Helical Propensity On Protein Folding, Stability, And Specificity*,” J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) “*The d'-d'-d' Vertical Triad is Less Discriminating Than the a'-a'-a' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif*,” J. Amer. Chem. Soc. 134(5):2626–2633; Straussman, R. *et al.* (2007) “*Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface*,” J. Molec. Biol. 366:1232-1242; Tripet, B. *et al.* (2002) “*Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance*,” J. Molec. Biol. 323:345–362; Woolfson, D.N. (2005) “*The Design Of Coiled-Coil Structures And Assemblies*,” Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) “*A Ligand-Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism*,” J. Gene Med. 10:355-367).

[00246] Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimer-Promoting Domain of the first polypeptide chain may comprise a sequence of eight negatively charged amino acid residues and the Heterodimer-Promoting Domain of the second polypeptide chain may comprise a sequence of eight negatively charged amino acid residues. It is

immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* The positively charged amino acid is preferably lysine and/or the negatively charged amino acid is preferably glutamic acid. It is possible for only a single Heterodimer-Promoting Domain to be employed (since such domain will inhibit homodimerization and thereby promote heterodimerization), however, it is preferred for both the first and second polypeptide chains of the diabodies of the present invention to contain Heterodimer-Promoting Domains.

[00247] In a preferred embodiment, one of the Heterodimer-Promoting Domains will comprise four tandem “E-coil” helical domains (SEQ ID NO:39: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem “K-coil” domains (SEQ ID NO:40: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimerization. Especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem “E-coil” helical domains of SEQ ID NO:39 has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:41). Likewise, especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem “K-coil” helical domains of SEQ ID NO:40 has been modified to contain a cysteine residue: KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:42).

[00248] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and

thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) “*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Modules*,” J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:43**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00249] As disclosed in WO 2012/162068 (herein incorporated by reference), “**deimmunized**” variants of **SEQ ID NO:43** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized albumin-binding domain: 66S/70S +71A; 66S/70S +79A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNA₆₄A₆₅NNAKT VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO: 44**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLIS₆₆NAKS₇₀ VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO: 45**),

are particularly preferred as such deimmunized Albumin-Binding Domains exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an Albumin-Binding Domain contains a third linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the Albumin-Binding Domain (which is preferably a

deimmunized Albumin-Binding Domain). A preferred sequence for such Linker 3 is **SEQ ID NO:46**: GGGS.

[00250] Thus, in sum, a preferred first polypeptide chain of a preferred Tri-Specific Binding Molecule of the present invention will comprise the Domains and linkers: **(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(E-coil Heterodimer-Promoting Domain)—(Linker 3)—(Knob-Bearing CH2-CH3 Domain)**.

3. Alternative First Polypeptide Chain

[00251] In one embodiment, the orientations of the above-described Domains will be in the N-terminal to C-terminal direction. The present invention, however, also contemplates a variation thereof, wherein the orientations of the Domains of the first polypeptide chain are: **NH₂-(Knob-Bearing CH3-CH2 Domain)-(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(E-coil Heterodimer-Promoting Domain)**. Preferably, a cysteine-containing peptide is present, N-terminal to such CH2-CH3 Domain. The sequence of an exemplary peptide is sequence (**SEQ ID NO:48**): DKTHTCPPCP. Preferably in this embodiment, the CH3 Domain is spaced apart from the VL_I Domain by an intervening peptide linker (Linker 4), such as one having the amino acid sequence of (**SEQ ID NO:152**): APSSS, and more preferably, the amino acid sequence (**SEQ ID NO:50**): APSSSPME.

4. Preferred Second Polypeptide Chain

[00252] A second polypeptide chain of such preferred Tri-Specific Binding Molecules will comprise, in the N-terminal to C-terminal direction, a Variable Light Chain Domain capable of binding to Epitope II (VL_{II}), a Variable Heavy Chain Domain capable of binding to Epitope I (VH_I), and a Heterodimer-Promoting Domain.

[00253] Since the Variable Light Chain and Variable Heavy Chain Domains of the second polypeptide are directed toward different epitopes, they cannot associate together to form a Binding Domain that is able to bind either Epitope I or Epitope II. The Variable Light Chain and Variable Heavy Chain Domains of the second polypeptide are spaced apart from one another by an intervening linker peptide that is

sufficiently short as to substantially prevent the association of these Domains. “**Linker 1**,” having the sequence (**SEQ ID NO:33**): GGGSGGGG is an exemplary linker for this purpose.

[00254] As in the case of the first polypeptide chain, the Variable Heavy Chain Domain of the second polypeptide and the Heterodimer-Promoting Domain of that polypeptide are preferably spaced apart from one another by an intervening linker peptide that contains 1, 2, 3 or more cysteine residues. “**Linker 2**,” having the sequence (**SEQ ID NO:34**) GGCGGG is an exemplary linker for this purpose. Such cysteine residues can form disulfide bonds with cysteine residues in the cysteine-containing spacer peptide that separates the Variable Heavy Chain Domain of the first polypeptide and the Heterodimer-Promoting Domain of that polypeptide. Thus, the first and second polypeptides of the Binding Molecules of the present invention are covalently bonded to one another.

[00255] As discussed above, the Heterodimer-Promoting Domain of the second polypeptide chain is selected so as coordinate with the Heterodimer-Promoting Domain of the first polypeptide chain. Thus, in a preferred embodiment, the Heterodimer-Promoting Domain of the first polypeptide chain is either a “**K-coil**” Domain (**SEQ ID NO:40**) or an “**E-coil**” Domain (**SEQ ID NO:39**). If the cysteine-containing E-coil (**SEQ ID NO:41**) is employed in the first polypeptide chain, then the cysteine-containing K-coil (**SEQ ID NO:42**) is preferably employed in the second polypeptide chain. Conversely, if the cysteine-containing K-coil (**SEQ ID NO:42**) is employed in the first polypeptide chain, then the cysteine-containing E-coil (**SEQ ID NO:41**) is preferably employed in the second polypeptide chain. Since the first polypeptide chain will preferably possess an “**E-coil**” Domain, the second polypeptide chain will preferably contain a “**K-coil**” Domain.

[00256] As the first and second polypeptide chains are polypeptide chains of a diabody, they are able to associate together to form a Domain I Binding Domain (VL_A/VH_A) that recognizes and immunospecifically binds to Epitope I, and a Domain II Binding Domain (VL_B/VH_B) that recognizes and immunospecifically binds to Epitope II.

[00257] Thus, in sum, a preferred second polypeptide chain of a preferred Binding Molecule of the present invention will comprise the Domains and linkers: **(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(K-coil Heterodimer-Promoting Domain)**.

5. Preferred Third Polypeptide Chain

[00258] A third polypeptide chain of a preferred Binding Molecule of the present invention is a polypeptide that comprises, in the N-terminal to C-terminal direction, a Binding Domain, an optional CH1-Hinge Domain, and a CH2-CH3 Domain. The Binding Domain of the third polypeptide chain of a preferred Binding Molecule of the present invention may be a Variable Heavy Chain Domain capable of binding to Epitope III (**VH_{III}**), in which case, the fourth polypeptide chain of the preferred Binding Molecules of the present invention (discussed below) is a polypeptide that comprises a Variable Light Chain Domain capable of binding to Epitope III (**VL_{III}**), such that the Binding Domain is capable of immunospecific binding to an antigen possessing Epitope III. Alternatively, the Binding Domain of the third polypeptide chain of the preferred Binding Molecules of the present invention may comprise an Effector Cell Receptor-Type Binding Domain, in which case, the fourth polypeptide chain of the preferred Binding Molecules of the present invention (discussed below) is a polypeptide that comprises a complementary Effector Cell Receptor-Type Binding Domain, such that the interaction of two polypeptide chains forms a Binding Domain that is capable of physiospecific binding to molecule present on the surface of the effector cell. The third polypeptide chain may be isolated from naturally occurring antibodies. Alternatively, it may be constructed recombinantly. An exemplary CH1 Domain is a human IgG1 CH1 Domain having the amino acid sequence (**SEQ ID NO:207**):

```
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKKV
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[00259] A variant of the human IgG1 CH1 Domain of SEQ ID NO:207 is (**SEQ ID NO:208**):

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ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKRV
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[00260] An exemplary Hinge Domain is a human IgG1 Hinge Domain having the amino acid sequence (**SEQ ID NO:209**): EPKSCDKTHTCPPCP. As will be recognized, the exemplary Hinge Domain comprises multiple cysteine residues (Elkabetz *et al.* (2005) “*Cysteines In CH1 Underlie Retention Of Unassembled Ig Heavy Chains*,” J. Biol. Chem. 280:14402-14412) that may participate in interchain covalent bonding.

[00261] Although a wild-type CH2-CH3 Domain may be employed, it is preferred, as described above, to employ a modified CH2-CH3 Domain that promotes heterodimerization with the CH2-CH3 Domain of the first polypeptide chain.

[00262] Preferably, therefore the CH2-CH3 Domain of the third polypeptide chain will be a “hole-bearing” CH2-CH3 Domain whose amino acid sequence is complementary to the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**) employed in the first polypeptide. As discussed above, the “hole-bearing” CH2-CH3 domain preferably should comprise a substitution at position 435 (H435R) to remove the Protein A binding site. An exemplary “hole-bearing” CH2-CH3 Domain with the H435R substitution for the third polypeptide is **SEQ ID NO:53**.

[00263] As will be recognized, a “knob-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:52**) could be employed in the third polypeptide chain, in which case, a “hole-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:53**) would be employed in the first polypeptide chain.

[00264] In the embodiment in which the third (and fourth) polypeptide chains of the preferred Tri-Specific Binding Molecules of the present invention each comprise a polypeptide chain of an Effector Cell Receptor-Type Binding Domain, methods for producing such Effector Cell Receptor-Type Binding Domains are well-known (*e.g.*, US2012/0294874A1).

[00265] Thus, in sum, a third polypeptide chain of the preferred Binding Molecules of the present invention will comprise the Domains and linkers: (**VH_{III} Domain**)—(**Optional CH1 Domain**)—(**Optional Hinge Domain**)—(**“Hole-Bearing” CH2-CH3 Domain**), or (**T Cell Receptor-Type Binding Domain**; first or

second polypeptide thereof)—(Optional CH1 Domain)—(Optional Hinge Domain)—(“Hole-Bearing” CH2-CH3 Domain).

6. Preferred Fourth Polypeptide Chain

[00266] A fourth polypeptide chain of the preferred Tri-Specific Binding Molecules of the present invention is either a polypeptide of an Effector Cell Receptor-Type Binding Domain (wherein the third and fourth polypeptides form a ligand for a receptor found on the surface of an effector cell, or more preferably, a light chain of the above-indicated antibody that immunospecifically binds to Epitope III or which are complementary to the binding domain of the third polypeptide chain.

[00267] Thus, wherein the third and fourth polypeptides form a Fab-Type Binding Domain such fourth polypeptide chain comprises, in the N-terminal to C-terminal direction, a Variable Light Chain Domain capable of binding to Epitope III (VL_{III}), and a Domain for promoting covalent bonding to the third polypeptide chain or a Binding Domain and such Domain for promoting covalent bonding to the third polypeptide chain. Such Domain may be a CL Domain, or a cysteine-containing portion thereof, such as (SEQ ID NO:38) FNRGEC or a linker such as Linker 2 (having the sequence (SEQ ID NO:34) GGCGGG. An exemplary a cysteine-containing peptide that forms disulfide bonds with such Linker 2 comprises the amino acid sequence VEPKSC (SEQ ID NO:36) or a Hinge Domain.

[00268] The fourth polypeptide chain may be isolated from naturally occurring antibodies. Alternatively, it may be constructed recombinantly. A preferred CL Domain is a human IgG1 CL Kappa Domain having the amino acid sequence (SEQ ID NO:210):

```
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC
```

[00269] Alternatively, an exemplary CL Domain is a human IgG1 CL Lambda2 Domain having the amino acid sequence (SEQ ID NO:211):

```
QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVP
TECS
```

[00270] As will be noticed, the CL Domain, or other Cysteine-Containing Domain, of the fourth polypeptide chain comprises cysteine residues. Such cysteine residues are able to covalently bond to cysteine residues of the CH1 Domain of the third polypeptide chain to thereby covalently complex the third and fourth polypeptide chains of the binding molecules of the present invention to one another. Thus the third and fourth polypeptide chains are covalently bonded to one another.

[00271] Additionally, cysteine residues of the CH2-CH3 Domain of the first polypeptide chain can form disulfide bonds with cysteine residues of the CH2-CH3 Domain of the third polypeptide chain. Thus the first and third polypeptide chains are covalently bonded to one another.

E. Variant Fc Domains

[00272] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody-dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Domain of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. The amino acid sequence of an exemplary IgG1 Fc Domain (SEQ ID NO:1) is presented above.

[00273] Modification of the Fc Domain normally leads to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. Reduction or elimination of effector function is desirable in certain cases, for example in the case of antibodies whose

mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*e.g.*, non-Hodgkins lymphoma, CLL, and Burkitt's lymphoma). In said embodiments, molecules of the invention with conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection where an enhanced efficacy of effector function activity is desired.

[00274] In certain embodiments, the Tri-Specific Binding Molecules of the present invention comprise an Fc Domain that possesses one or more modifications (*e.g.*, substitutions, deletions, or insertions) to the sequence of amino acids of a wild-type Fc Domain (**SEQ ID NO:1**), which reduce the affinity and avidity of the Fc Domain and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules of the invention comprise an Fc Domain that possesses one or more modifications to the amino acids of the wild-type Fc Domain, which increase the affinity and avidity of the Fc Domain and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules comprise a variant Fc Domain wherein said variant confers or mediates increased ADCC activity and/or an increased binding to FcγRIIA, relative to a molecule comprising no Fc Domain or comprising a wild-type Fc Domain. In alternate embodiments, the molecules comprise a variant Fc Domain wherein said variant confers or mediates decreased ADCC activity (or other effector function) and/or an increased binding to FcγRIIB, relative to a molecule comprising no Fc Domain or comprising a wild-type Fc Domain. In some embodiments, the invention encompasses Tri-Specific Binding Molecules comprising a variant Fc Domain, which variant Fc Domain does not show a detectable binding to any FcγR, relative to a comparable molecule comprising the wild-type Fc Domain. In other embodiments, the invention encompasses Tri-Specific Binding Molecules comprising a variant Fc Domain, which variant Fc Domain only binds a single FcγR, preferably one of FcγRIIA, FcγRIIB, or FcγRIIIA. Any such increased affinity and/or avidity is preferably assessed by measuring *in vitro* the extent of detectable binding to the FcγR or FcγR-related activity in cells that express low levels of the FcγR when binding

activity of the parent molecule (without the modified Fc Domain) cannot be detected in the cells, or in cells which express non-FcγR receptor target antigens at a density of 30,000 to 20,000 molecules/cell, at a density of 20,000 to 10,000 molecules/cell, at a density of 10,000 to 5,000 molecules/cell, at a density of 5,000 to 1,000 molecules/cell, at a density of 1,000 to 200 molecules/cell or at a density of 200 molecules/cell or less (but at least 10, 50, 100 or 150 molecules/cell).

[00275] The Tri-Specific Binding Molecules of the present invention may comprise altered affinities for an activating and/or inhibitory Fcγ receptor. In one embodiment, the Tri-Specific Binding Molecule comprises a variant Fc Domain that has increased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Domain. In another embodiment, the Tri-Specific Binding Molecule of the present invention comprise a variant Fc Domain, which has decreased affinity for FcγRIIB and increased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Domain. In yet another embodiment, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain that has decreased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Domain. In still another embodiment, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain, which has unchanged affinity for FcγRIIB and decreased (or increased) affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Domain.

[00276] In certain embodiments, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain having an altered affinity for FcγRIIIA and/or FcγRIIA such that the immunoglobulin has an enhanced effector function, e.g., antibody-dependent cell-mediated cytotoxicity. Non-limiting examples of effector cell functions include antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis, phagocytosis, opsonization, opsonophagocytosis, cell binding, rosetting, C1q binding, and complement dependent cell-mediated cytotoxicity.

[00277] In a preferred embodiment, the alteration in affinity or effector function is at least 2-fold, preferably at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 50-fold, or at least 100-fold, relative to a comparable molecule comprising a wild-type Fc Domain. In other embodiments of the invention, the variant Fc Domain immunospecifically binds one or more FcRs with at least 65%, preferably at least 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 225%, or 250% greater affinity relative to a molecule comprising a wild-type Fc Domain. Such measurements can be in vivo or in vitro assays, and in a preferred embodiment are in vitro assays such as ELISA or surface plasmon resonance assays.

[00278] In different embodiments, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain wherein said variant agonizes at least one activity of an FcγR receptor, or antagonizes at least one activity of an FcγR receptor. In a preferred embodiment, the molecules comprise a variant that antagonizes one or more activities of FcγRIIB, for example, B cell receptor-mediated signaling, activation of B cells, B cell proliferation, antibody production, intracellular calcium influx of B cells, cell cycle progression, FcγRIIB-mediated inhibition of FcεRI signaling, phosphorylation of FcγRIIB, SHIP recruitment, SHIP phosphorylation and association with Shc, or activity of one or more downstream molecules (e.g., MAP kinase, JNK, p38, or Akt) in the FcγRIIB signal transduction pathway. In another embodiment, the Tri-Specific Binding Molecules of the present invention comprise a variant that agonizes one or more activities of FcεRI, for example, mast cell activation, calcium mobilization, degranulation, cytokine production, or serotonin release.

[00279] In certain embodiments, the molecules comprise an Fc Domain comprising regions from two or more IgG isotypes (e.g., IgG1, IgG2, IgG3 and IgG4). The various IgG isotypes exhibit differing physical and functional properties including serum half-life, complement fixation, FcγR binding affinities and effector function activities (e.g., ADCC, CDC, *etc.*) due to differences in the amino acid sequences of their hinge and/or Fc Domains, for example as described in Flesch and Neppert (1999) J. Clin. Lab. Anal. 14:141-156; Chappel *et al.* (1993) J. Biol. Chem. 33:25124-

25131; Chappel *et al.* (1991) *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9036-9040; or Brüggemann *et al.* (1987) *J. Exp. Med* 166:1351-1361. This type of variant Fc Domain may be used alone, or in combination with an amino acid modification, to affect Fc-mediated effector function and/or binding activity. In combination, the amino acid modification and IgG hinge/Fc Domain may display similar functionality (e.g., increased affinity for FcγRIIA) and may act additively or, more preferably, synergistically to modify the effector functionality in the molecule of the invention, relative to a molecule of the invention comprising a wild-type Fc Domain. In other embodiments, the amino acid modification and IgG Fc Domain may display opposite functionality (e.g., increased and decreased affinity for FcγRIIA, respectively) and may act to selectively temper or reduce a specific functionality in the molecule of the invention, relative to a molecule of the invention not comprising an Fc Domain or comprising a wild-type Fc Domain of the same isotype.

[00280] In a preferred specific embodiment, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain, wherein said variant Fc Domain comprises at least one amino acid modification relative to a wild-type Fc Domain, such that said molecule has an altered affinity for an FcR, provided that said variant Fc Domain does not have a substitution at positions that make a direct contact with FcγR based on crystallographic and structural analysis of Fc-FcR interactions such as those disclosed by Sondermann *et al.* (2000) *Nature* 406:267-73. Examples of positions within the Fc Domain that make a direct contact with FcγR are amino acid residues 234-239 (hinge region), amino acid residues 265-269 (B/C loop), amino acid residues 297-299 (C'/E loop), and amino acid residues 327-332 (F/G loop). In some embodiments, the molecules of the invention comprise variant Fc Domains comprise modification of at least one residue that does not make a direct contact with an FcγR based on structural and crystallographic analysis, e.g., is not within the Fc-FcγR binding site.

[00281] Variant Fc Domains are well-known in the art, and any known Fc variant may be used in the present invention to confer or modify the effector function exhibited by a molecule of the invention comprising an Fc Domain (or portion thereof) as functionally assayed, e.g., in an NK dependent or macrophage dependent

assay. For example, Fc Domain variants identified as altering effector function are disclosed in the Antibody Engineering Technology Art, and any suitable variant disclosed therein may be used in the present molecules.

[00282] In certain embodiments, the Tri-Specific Binding Molecules of the present invention comprise a variant Fc Domain, having one or more amino acid modifications in one or more sites, which modification(s) alter (relative to a wild-type Fc Domain) the **Ratio of Affinities** of the variant Fc Domain to an activating FcγR (such as FcγRIIA or FcγRIIIA) relative to an inhibiting FcγR (such as FcγRIIB):

$$\text{Ratio of Affinities} = \frac{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Activating}}}{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Inhibiting}}}$$

[00283] Particularly preferred are Tri-Specific Binding Molecules of the present invention that possess a variant Fc Domain (relative to the wild-type Fc Domain) in which the Fc variant has a Ratio of Affinities greater than 1. Such molecules have particular use in providing a therapeutic or prophylactic treatment of a disease, disorder, or infection, or the amelioration of a symptom thereof, where an enhanced efficacy of effector cell function (e.g., ADCC) mediated by FcγR is desired, e.g., cancer or infectious disease. In contrast, an Fc variant having a Ratio of Affinities less than 1 mediates decreased efficacy of effector cell function. **Table 1** lists exemplary single, double, triple, quadruple and quintuple mutations by whether their Ratio of Affinities is greater than or less than 1.

Table 1				
Exemplary Single and Multiple Mutations Listed by Ratio of Affinities				
Single	Double	Triple	Quadruple	Quintuple
Ratio of Affinities > 1				
F243L D270E R292G R292P	F243L & R292P	F243L, P247L & N421K	L234F, F243L, R292P & Y300L	L235V, F243L, R292P, Y300L & P396L
	F243L & Y300L	F243L, R292P & Y300L	L235I, F243L, R292P & Y300L	L235P, F243L, R292P, Y300L & P396L
	F243L & P396L	F243L, R292P & V305I	L235Q, F243L, R292P & Y300L	F243L, R292P, V305I, Y300L & P396L
	D270E & P396L	F243L, R292P & P396L	F243L, P247L, D270E & N421K	
	R292P & Y300L	F243L, Y300L & P396L	F243L, R255L, D270E & P396L	

Table 1				
Exemplary Single and Multiple Mutations Listed by Ratio of Affinities				
Single	Double	Triple	Quadruple	Quintuple
Ratio of Affinities > 1				
	R292P & V305I	P247L, D270E & N421K	F243L, D270E, G316D & R416G	
	R292P & P396L	R255L, D270E & P396L	F243L, D270E, K392T & P396L	
	Y300L & P396L	D270E, G316D & R416G	F243L, D270E, P396L & Q419H	
	P396L & Q419H	D270E, K392T & P396L	F243L, R292P, Y300L, & P396L	
		D270E, P396L & Q419H	F243L, R292P, V305I & P396L	
		V284M, R292L & K370N	P247L, D270E, Y300L & N421K	
		R292P, Y300L & P396L	R255L, D270E, R292G & P396L	
			R255L, D270E, Y300L & P396L	
			D270E, G316D, P396L & R416G	
Ratio of Affinities < 1				
Y300L P396L	F243L & P396L P247L & N421K R255L & P396L R292P & V305I K392T & P396L P396L & Q419H	F243L, R292P & V305I		

[00284] In a specific embodiment, in variant Fc Domains, any amino acid modifications (e.g., substitutions) at any of positions 235, 240, 241, 243, 244, 247, 262, 263, 269, 298, 328, or 330 and preferably one or more of the following residues: A240, I240, L241, L243, H244, N298, I328 or V330. In a different specific embodiment, in variant Fc Domains, any amino acid modifications (e.g., substitutions) at any of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376,

416, 419, 430, 434, 435, 437, 438 or 439 and preferably one or more of the following residues: H280, Q280, Y280, G290, S290, T290, Y290, N294, K295, P296, D298, N298, P298, V298, I300 or L300.

[00285] In a preferred embodiment, in variant Fc Domains that bind an Fc γ R with an altered affinity, any amino acid modifications (e.g., substitutions) at any of positions 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439. Preferably, the variant Fc Domain has any of the following residues: A256, N268, Q272, D286, Q286, S286, A290, S290, A298, M301, A312, E320, M320, Q320, R320, E322, A326, D326, E326, N326, S326, K330, T339, A333, A334, E334, H334, L334, M334, Q334, V334, K335, Q335, A359, A360 or A430.

[00286] In a different embodiment, in variant Fc Domains that bind an Fc γ R (via its Fc Domain) with a reduced affinity, any amino acid modifications (e.g., substitutions) at any of positions 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438 or 439.

[00287] In a different embodiment, in variant Fc Domains that bind an Fc γ R (via its Fc Domain) with an enhanced affinity, any amino acid modifications (e.g., substitutions) at any of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398 or 430. In a different embodiment, in variant Fc Domains that binds Fc γ RIIA with an enhanced affinity, any of the following residues: A255, A256, A258, A267, A268, N268, A272, Q272, A276, A280, A283, A285, A286, D286, Q286, S286, A290, S290, M301, E320, M320, Q320, R320, E322, A326, D326, E326, S326, K330, A331, Q335, A337 or A430.

[00288] Preferred variants include one or more modifications at any of positions: 228, 230, 231, 232, 233, 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264,

265, 266, 271, 273, 275, 281, 284, 291, 296, 297, 298, 299, 302, 304, 305, 313, 323, 325, 326, 328, 330 or 332.

[00289] Particularly preferred variants include one or more modifications selected from groups A-AI:

A	228E, 228K, 228Y or 228G;
B	230A, 230E, 230Y or 230G;
C	231E, 231K, 231Y, 231P or 231G;
D	232E, 232K, 232Y, 232G;
E	233D;
F	234I or 234F;
G	235D, 235Q, 235P, 235I or 235V;
H	239D, 239E, 239N or 239Q;
I	240A, 240I, 240M or 240T;
J	243R, 243, 243Y, 243L, 243Q, 243W, 243H or 243I;
K	244H;
L	245A;
M	247G, 247V or 247L;
N	262A, 262E, 262I, 262T, 262E or 262F;
O	263A, 263I, 263M or 263T;
P	264F, 264E, 264R, 264I, 264A, 264T or 264W;
Q	265F, 265Y, 265H, 265I, 265L, 265T, 265V, 265N or 265Q;
R	266A, 266I, 266M or 266T;
S	271D, 271E, 271N, 271Q, 271K, 271R, 271S, 271T, 271H, 271A, 271V, 271L, 271I, 271F, 271M, 271Y, 271W or 271G;
T	273I;
U	275L or 275W;
V	281D, 281K, 281Y or 281P;
W	284E, 284N, 284T, 284L, 284Y or 284M;
X	291D, 291E, 291Q, 291T, 291H, 291I or 291G;
Y	299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W or 299Y;
Z	302I;
AA	304D, 304N, 304T, 304H or 304L
AB	305I;
AC	313F;
AD	323I;
AE	325A, 325D, 325E, 325G, 325H, 325I, 325L, 325K, 325R, 325S, 325F, 325M, 325T, 325V, 325Y, 325W or 325P;
AF	328D, 328Q, 328K, 328R, 328S, 328T, 328V, 328I, 328Y, 328W, 328P, 328G, 328A, 328E, 328F, 328H, 328M or 328N;
AG	330L, 330Y, 330I or 330V;
AH	332A, 332D, 332E, 332H, 332N, 332Q, 332T, 332K, 332R, 332S, 332V, 332L, 332F, 332M, 332W, 332P, 332G or 332Y; and
AI	336E, 336K or 336Y

[00290] Still more particularly preferred variants include one or more modifications selected from Groups 1-105:

Group	Variant	Group	Variant
1	A330L / I332E	54	S239D / D265L / N297D / I332E
2	D265F / N297E / I332E	55	S239D / D265T / N297D / I332E

3	D265Y / N297D / I332E	56	S239D / D265V / N297D / I332E
4	D265Y / N297D / T299L / I332E	57	S239D / D265Y / N297D / I332E
5	F241E / F243Q / V262T / V264F	58	S239D / I332D
6	F241E / F243Q / V262T / V264E / I332E	59	S239D / I332E
7	F241E / F243R / V262E / V264R	60	S239D / I332E / A330I
8	F241E / F243R / V262E / V264R / I332E	61	S239D / I332N
9	F241E / F243Y / V262T / V264R	62	S239D / I332Q
10	F241E / F243Y / V262T / V264R / I332E	63	S239D / N297D / I332E
11	F241L / F243L / V262I / V264I	64	S239D / N297D / I332E / A330Y
12	F241L / V262I	65	S239D / N297D / I332E / A330Y / F241S / F243H / V262T / V264T
13	F241R / F243Q / V262T / V264R	66	S239D / N297D / I332E / K326E
14	F241R / F243Q / V262T / V264R / I332E	67	S239D / N297D / I332E / L235D
15	F241W / F243W / V262A / V264A	68	S239D / S298A / I332E
16	F241Y / F243Y / V262T / V264T	69	S239D / V264I / A330L / I332E
17	F241Y / F243Y / V262T / V264T / N297D / I332E	70	S239D / V264I / I332E
18	F243L / V262I / V264W	71	S239D / V264I / S298A / I332E
19	P243L / V264I	72	S239E / D265N
20	L328D / I332E	73	S239E / D265Q
21	L328E / I332E	74	S239E / I332D
22	L328H / I332E	75	S239E / I332E
23	L328I / I332E	76	S239E / I332N
24	L328M / I332E	77	S239E / I332Q
25	L328N / I332E	78	S239E / N297D / I332E
26	L328Q / I332E	79	S239E / V264I / A330Y / I332 E
27	L328T / I332E	80	S239E / V264I / I332 E
28	L328V / I332E	81	S239E / V264I / S298A / A330Y / I332E
29	N297D / A330Y / I332E	82	S239N / A330L / I332E
30	N297D / I332E	83	S239N / A330Y / I332E
31	N297D / I332E / S239D / A330L	84	S239N / I332D
32	N297D / S298A / A330Y / I 332E	85	S239N / I332E
33	N297D / T299L / I332E	86	S239N / I332N
34	N297D / T299F / I332E / N297D / T299H / I332E	87	S239N / I332Q
35	N297D / T299I / I332E	88	S239N1S298A / I332E
36	N297D / T299L / I332E	89	S239Q / I332D
37	N297D / T299V / I332E	90	S239Q / I332E
38	N297E / I332E	91	S239Q / I332N
39	N297S / I332E	92	S239Q / I332Q
40	P230A / E233D / I332E	93	S239Q / V264I / I332E
41	P244H / P245A / P247V	94	S298A / I332E
42	S239D / A330L / I332E	95	V264E / N297D / I332E

43	S239D / A330Y / I332E	96	V264I / A330L / I332E
44	S239D / A330Y / I332E / K326E	97	V264I / A330Y / I332E
45	S239D / A330Y / I332E / K326T	98	V264I / I332E
46	S239D / A330Y / I332E / L234I	99	V264I / S298A / I332E
47	S239D / A330Y / I332E / L235D	100	Y296D / N297D / I332E
48	S239D / A330Y / I332E / V240I	101	Y296E / N297D / I332 E
49	S239D / A330Y / I332E / V264T	102	Y296H / N297D / I332E
50	S239D / A330Y / I332E / V266I	103	Y296N / N297D / I332E
51	S239D / D265F / N297D / I332E	104	Y296Q / N297I / I332E
52	S239D / D265H / N297D / I332E	105	Y296T / N297D / I332E.
53	S239D / D265I / N297D / I332E		

[00291] In one embodiment, a multivalent DR5 binding molecule of the invention will comprise a variant Fc Domain having at least one modification in the Fc Domain. In certain embodiments, the variant Fc Domain comprises at least one substitution selected from the group consisting of L235V, F243L, R292P, Y300L, V305I, and P396L, wherein said numbering is that of the EU index as in Kabat.

[00292] In a specific embodiment, the variant Fc Domain comprises:

- (A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;
- (B) at least two substitutions selected from the group consisting of:
 - (1) F243L and P396L;
 - (2) F243L and R292P; and
 - (3) R292P and V305I;
- (C) at least three substitutions selected from the group consisting of:
 - (1) F243L, R292P and Y300L;
 - (2) F243L, R292P and V305I;
 - (3) F243L, R292P and P396L; and
 - (4) R292P, V305I and P396L;
- (D) at least four substitutions selected from the group consisting of:
 - (1) F243L, R292P, Y300L and P396L; and
 - (2) F243L, R292P, V305I and P396L; or
- (E) at least the five substitutions selected from the group consisting of:
 - (1) F243L, R292P, Y300L, V305I and P396L; and
 - (2) L235V, F243L, R292P, Y300L and P396L.

[00293] In another specific embodiment, the variant Fc Domain comprises substitutions of:

- (A) F243L, R292P, and Y300L;
- (B) L235V, F243L, R292P, Y300L, and P396L; or
- (C) F243L, R292P, Y300L, V305I, and P396L.

[00294] In other embodiments, the invention encompasses the use of any Fc variant known in the art, such as those disclosed in Jefferis, B.J. *et al.* (2002) “*Interaction Sites On Human IgG-Fc For FcγR: Current Models*,” Immunol. Lett. 82:57-65; Presta, L.G. *et al.* (2002) “*Engineering Therapeutic Antibodies For Improved Function*,” Biochem. Soc. Trans. 30:487-90; Idusogie, E.E. *et al.* (2001) “*Engineered Antibodies With Increased Activity To Recruit Complement*,” J. Immunol. 166:2571-75; Shields, R.L. *et al.* (2001) “*High Resolution Mapping Of The Binding Site On Human IgG1 For Fc γR1, Fc γR2, Fc γR3, And FcγR4 And Design Of IgG1 Variants With Improved Binding To The Fc γR*,” J. Biol. Chem. 276:6591-6604; Idusogie, E.E. *et al.* (2000) “*Mapping Of The C1q Binding Site On Rituxan, A Chimeric Antibody With A Human IgG Fc*,” J. Immunol. 164:4178-84; Reddy, M.P. *et al.* (2000) “*Elimination Of Fc Receptor-Dependent Effector Functions Of A Modified IgG4 Monoclonal Antibody To Human CD4*,” J. Immunol. 164:1925-1933; Xu, D. *et al.* (2000) “*In Vitro Characterization of Five Humanized OKT3 Effector Function Variant Antibodies*,” Cell. Immunol. 200:16-26; Armour, K.L. *et al.* (1999) “*Recombinant human IgG Molecules Lacking FcγR1 Binding And Monocyte Triggering Activities*,” Eur. J. Immunol. 29:2613-24; Jefferis, R. *et al.* (1996) “*Modulation Of Fc(γ)R And Human Complement Activation By IgG3-Core Oligosaccharide Interactions*,” Immunol. Lett. 54:101-04; Lund, J. *et al.* (1996) “*Multiple Interactions Of IgG With Its Core Oligosaccharide Can Modulate Recognition By Complement And Human Fc γR1 And Influence The Synthesis Of Its Oligosaccharide Chains*,” J. Immunol. 157:4963-4969; Hutchins *et al.* (1995) “*Improved Biodistribution, Tumor Targeting, And Reduced Immunogenicity In Mice With A γ4 Variant Of Campath-1H*,” Proc. Natl. Acad. Sci. (U.S.A.) 92:11980-84; Jefferis, R. *et al.* (1995) “*Recognition Sites On Human IgG For Fc γ Receptors: The Role Of Glycosylation*,” Immunol. Lett. 44:111-17; Lund, J. *et al.* (1995) “*Oligosaccharide-Protein Interactions In IgG Can*

Modulate Recognition By Fc Gamma Receptors,” FASEB J. 9:115-19; Alegre, M.L. *et al.* (1994) “A Non-Activating “Humanized” Anti-CD3 Monoclonal Antibody Retains Immunosuppressive Properties In Vivo,” Transplantation 57:1537-1543; Lund *et al.* (1992) “Multiple Binding Sites On The CH2 Domain Of IgG For Mouse Fc Gamma R11,” Mol. Immunol. 29:53-59; Lund *et al.* (1991) “Human Fc Gamma RI And Fc Gamma RII Interact With Distinct But Overlapping Sites On Human IgG,” J. Immunol. 147:2657-2662; Duncan, A.R. *et al.* (1988) “Localization Of The Binding Site For The Human High-Affinity Fc Receptor On IgG,” Nature 332:563-564; US Patent Nos. 5,624,821; 5,885,573; 6,194,551; 7,276,586; and 7,317,091; and PCT Publications WO 00/42072 and PCT WO 99/58572.

[00295] In some embodiments, the molecules of the invention further comprise one or more glycosylation sites, so that one or more carbohydrate moieties are covalently attached to the molecule. Preferably, the molecules of the invention with one or more glycosylation sites and/or one or more modifications in the Fc Domain confer or have an enhanced antibody-mediated effector function, *e.g.*, enhanced ADCC activity, compared to a parent antibody. In some embodiments, the invention further comprises molecules comprising one or more modifications of amino acids that are directly or indirectly known to interact with a carbohydrate moiety of the antibody, including but not limited to amino acids at positions 241, 243, 244, 245, 245, 249, 256, 258, 260, 262, 264, 265, 296, 299, and 301. Amino acids that directly or indirectly interact with a carbohydrate moiety of an antibody are known in the art, *see, e.g.*, Jefferis *et al.*, 1995 *Immunology Letters*, 44: 111-7, which is incorporated herein by reference in its entirety.

[00296] In another embodiment, the invention encompasses molecules that have been modified by introducing one or more glycosylation sites into one or more sites of the molecules, preferably without altering the functionality of the molecules, *e.g.*, binding activity to target antigen or FcγR. Glycosylation sites may be introduced into the variable and/or constant region of the molecules of the invention. As used herein, “glycosylation sites” include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains

are typically linked to the backbone of an antibody via either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. The molecules of the invention may comprise one or more glycosylation sites, including N-linked and O-linked glycosylation sites. Any glycosylation site for N-linked or O-linked glycosylation known in the art may be used in accordance with the instant invention. An exemplary N-linked glycosylation site that is useful in accordance with the methods of the present invention is the amino acid sequence: Asn-X-Thr/Ser, wherein X may be any amino acid and Thr/Ser indicates a threonine or a serine. Such a site or sites may be introduced into a molecule of the invention using methods well-known in the art to which this invention pertains (see for example, *IN VITRO* MUTAGENESIS, RECOMBINANT DNA: A SHORT COURSE, J. D. Watson, *et al.* W.H. Freeman and Company, New York, 1983, chapter 8, pp. 106-116, which is incorporated herein by reference in its entirety. An exemplary method for introducing a glycosylation site into a molecule of the invention may comprise: modifying or mutating an amino acid sequence of the molecule so that the desired Asn-X-Thr/Ser sequence is obtained.

[00297] In some embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies (and molecules comprising antibody domains) are well-known in the art and encompassed within the invention, *see, e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In other embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by deleting one or more endogenous carbohydrate moieties of the molecule. In a specific embodiment, the invention encompasses shifting the glycosylation site of the Fc Domain of an antibody, by modifying positions adjacent to 297. In a specific embodiment, the invention encompasses modifying position 296 so that position 296 and not position 297 is glycosylated.

[00298] Effector function can also be modified by techniques such as by introducing one or more cysteine residues into the Fc Domain, thereby allowing interchain disulfide bond formation in this region to occur, resulting in the generation of a homodimeric antibody that may have improved internalization capability and/or increased complement-mediated cell killing and ADCC (Caron, P.C. *et al.* (1992) “*Engineered Humanized Dimeric Forms Of IgG Are More Effective Antibodies*,” J. Exp. Med. 176:1191-1195; Shopes, B. (1992) “*A Genetically Engineered Human IgG Mutant With Enhanced Cytolytic Activity*,” J. Immunol. 148(9):2918-2922. Homodimeric antibodies with enhanced antitumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff, E.A. *et al.* (1993) “*Monoclonal Antibody Homodimers: Enhanced Antitumor Activity In Nude Mice*,” Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc Domains and may thereby have enhanced complement lysis and ADCC capabilities (Stevenson, G.T. *et al.* (1989) “*A Chimeric Antibody With Dual Fc Domains (bisFabFc) Prepared By Manipulations At The IgG Hinge*,” Anti-Cancer Drug Design 3:219-230).

III. Exemplary Trispecific Binding Molecules

F. gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1

[00299] An exemplary Tri-Specific Binding Molecule composed of four polypeptide chains was constructed. The Tri-Specific Binding Molecule comprises the VL and VH domains of gpA33 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of DR5 mAb 1, and was accordingly designated “gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (SEQ ID NO:212):

```
DIQLTQSPSF LSASVGDRVIT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GPENNYKTT
```

PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PGK

[00300] In **SEQ ID NO:212**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (**SEQ ID NO:181**), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ ID NO:112**), residues 240-245 correspond to the GGC GGG linker (**SEQ ID NO:34**), residues 246-273 correspond to an E-coil Domain (**SEQ ID NO:39**), residues 274-276 are the linker GGG, residues 277-286 are the linker DKTHTCPPCP (**SEQ ID NO:48**), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**).

[00301] A polynucleotide that encodes **SEQ ID NO:212** is **SEQ ID NO:213**:

```

gacattcagc  tgactcagtc  cccctctttt  ctgtccgcat  ccgtcggaga
tcgagtgact  attacttgct  ctgctaggtc  ctcaatcagc  ttcattgtact
ggtatcagca  gaagcccggc  aaagcaccta  agctgctgat  ctacgacaca
agcaacctgg  cctccggggg  gccatctcgg  ttctctggca  gtgggtcagg
aactgagttt  accctgacaa  ttagctccct  ggaggctgaa  gatgccgcta
cctactattg  ccagcagtgg  agcagctatc  ctctgacctt  cggacagggg
actaaactgg  aaatcaaggg  tggaggatcc  ggcggcggag  gcgaggtgca
gctggtggag  tctgggggag  gcttggtcca  gcctggaggg  tccctgagac
tctcctgtgc  agcctctgga  ttcaccttca  gcacatacgc  tatgaattgg
gtccgccagg  ctccagggaa  ggggctggag  tgggttgga  ggatcaggtc
caagtacaac  aattatgcaa  cctactatgc  cgactctgtg  aagggtagat
tcaccatctc  aagagatgat  tcaaagaact  cactgtatct  gcaaataaac
agcctgaaaa  ccgaggacac  ggccgtgtat  tactgtgtga  gacacggtaa
cttcggcaat  tcttacgtgt  cttgggtttg  ttattgggga  caggggacac
tggtgactgt  gtcttcggga  ggatgtggcg  gtggagaagt  ggccgcactg
gagaaagagg  ttgctgcttt  ggagaaggag  gtcgctgcac  ttgaaaagga
ggtcgcagcc  ctggagaaa  ggcggcggga  caaaactcac  acatgccccc
cgtgcccagc  acctgaagcc  gcggggggac  cgtcagtcct  cctcttcccc
ccaaaaccca  aggacaccct  catgatctcc  cggacccttg  aggtcacatg
cgtggtggtg  gacgtgagcc  acgaagaccc  tgaggtaaac  ttcaactggt
acgtggacgg  cgtggaggtg  cataatgcca  agacaaagcc  gcgggaggag
cagtacaaca  gcacgtaccg  tgtggtcagc  gtccctcacc  tcctgcacca
ggactggctg  aatggcaagg  agtacaagtg  caaggtctcc  aacaaagccc
tcccagcccc  catcgagaaa  accatctcca  aagccaaagg  gcagccccga
gaaccacagg  tgtacaccct  gcccccatcc  cgggaggaga  tgaccaagaa
ccaggtcagc  ctgtggtgcc  tgggtcaaagg  cttctatccc  agcgacatcg
ccgtggagtg  ggagagcaat  gggcagccgg  agaacaacta  caagaccacg
cctcccgtgc  tggactccga  cggtccttc  ttctcttaca  gcaagctcac
cgtggacaag  agcagggtgg  agcaggggaa  cgtcttctca  tgctccgtga

```

tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaaa

[00302] The amino acid sequence of the second polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:214**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARIYGN NVYFDVWGQG TTVTVSSGGC GGGKVAALKE
KVAALKEKVA ALKEKVAALK E

[00303] In **SEQ ID NO:214**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (**SEQ ID NO:186**), residues 238-243 correspond to the linker GGCGGG (**SEQ ID NO:34**), and residues 244-271 are a K-coil Domain (**SEQ ID NO:40**).

[00304] A polynucleotide that encodes **SEQ ID NO:214** is (**SEQ ID NO:215**):

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccctggacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg
tggacaggtc cagctggtcc agagcggggc cgaagtcaaa aaacccgag
caagcgtgaa ggtctcctgc aaagcatcag gctatacatt tacaggcagc
tggatgaact gggtgaggca ggctccagga cagggactgg agtggatcgg
gcgcacatctac cctggagacg gcgaaactaa ctataatgga aagttcaaa
accgagtgac catcacagcc gataagtcta ctagtaccgc ctacatggag
ctgagctccc tgcggtctga agataccgcc gtctactatt gcgctagaat
ttacggaaac aatgtctatt ttgacgtgtg ggggcaggga acaactgtga
ctgtctcctc cggaggatgt ggcggtggaa aagtggccgc actgaaggag
aaagttgctg ctttgaaaga gaaggctgcc gcacttaagg aaaaggctgc
agccctgaaa gag

[00305] The amino acid sequence of the third polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:216**):

EVKFLESFGG LVQPGGSLKL SCVASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTTRA

YYGNPAWFAY WGQGTLLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
 KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
 TYICNVNHKP SNTKVDKRVE PKSCDKHTC PPCPAPEAAG GPSVFLFPPK
 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPSRE EMTKNQVSL SCAVKGFYPSD IAVEWESNGQ PENNYKTPP
 VLDSDGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNRY TQKSLSLSPG
 K

[00306] In **SEQ ID NO:216**, amino acid residues 1-121 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (**SEQ ID NO:8**), residues 122-219 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 220-234 correspond to a linker (**SEQ ID NO:209**), and residues 235-451 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00307] A polynucleotide that encodes **SEQ ID NO:216** is (**SEQ ID NO:217**):

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
 cctgaaactc tcctgtgtag cctcaggatt cgatttttagt agatactgga
 tgagttgggt ccggcaggct ccagggaaag ggctagaatg gattggagaa
 attaattccag atagcaatac gataaactat acgccatctc taaaggataa
 attcatcatc tccagagaca acgccaacaaa tacgctgtat ctgcaaatga
 ccaaagttag atctgaggac acagcccttt attattgtac aagaaggggc
 tactatggta acccggcctg gtttgcttac tggggccaag ggactctggt
 cactgtctct tccgcctcca ccaagggccc atcggctctc cccctggcac
 cctcctccaa gagcacctct gggggcacag cggccctggg ctgcctggtc
 aaggactact tccccgaacc ggtgacggtg tcgtggaact caggcgcctt
 gaccagcggc gtgcacacct tcccggtgtt cctacagtcc tcaggactct
 actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag
 acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa
 gagagttgag cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc
 cagcacctga agccgcgggg ggaccgtcag tcttcctctt cccccaaaa
 cccaaggaca ccctcatgat ctcccgacc cctgaggtca catgctgtgt
 ggtggacgtg agccacgaag accctgaggt caagttcaac tggtagctgg
 acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac
 aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg
 gctgaatggc aaggagtaca agtgcaagggt ctccaacaaa gccctcccag
 ccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
 caggtgtaca ccctgcccc atcccgagg gagatgacca agaaccaggt
 cagcctgagt tgcgcagtca aaggcttcta tcccagcgac atcgccgtgg
 agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc
 gtgctggact ccgacggctc cttcttcctc gtcagcaagc tcaccgtgga
 caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg
 aggctctgca caaccgctac acgcagaaga gcctctccct gtctccgggt
 aaa

[00308] The amino acid sequence of the fourth polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:218**):

```
DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLs STLTLKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC
```

[00309] In **SEQ ID NO:218**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), and residues 112-218 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00310] A polynucleotide that encodes **SEQ ID NO:218** is (**SEQ ID NO:219**):

```
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcactggtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtgagg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaacgtacgg tggctgcacc
atcggctctc atcttcccgc catctgatga gcagttgaaa tctggaactg
cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta
cagtggaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt
cacagagcag gacagcaagg acagcaccta cagcctcagc agcaccctga
cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga
gtgt
```

G. gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 2

[00311] A second exemplary Tri-Specific Binding Molecule composed of four polypeptide chains was constructed. The Tri-Specific Binding Molecule comprises the VL and VH domains of gpA33 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of DR5 mAb 2, and was accordingly designated “gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 2.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:220**):

```
DIQLTQSPSF LSASVGDRVt ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLTVVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
```

PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
 PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
 PGK

[00312] In **SEQ ID NO:220**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (**SEQ ID NO:181**), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ ID NO:112**), residues 240-245 correspond to the GGCGGG linker (**SEQ ID NO:34**), residues 246-273 correspond to an E-coil Domain (**SEQ ID NO:39**), residues 274-276 are the linker GGG, residues 277-286 are the linker DKHTTCPPCP (**SEQ ID NO:48**), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**).

[00313] A polynucleotide that encodes **SEQ ID NO:220** is (**SEQ ID NO:221**):

gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
 tcgagtgact attacttgct ctgctagggtc ctcaatcagc ttcatgtact
 ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
 agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
 aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
 cctactattg ccagcagtg agcagctatc ctctgacctt cggacagggg
 actaaactgg aaatcaaggg tggaggatcc ggcggcggag gcgagggtgca
 gctggtggag tctgggggag gcttggtcca gcctggaggg tccttgagac
 tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
 gtccgccagg ctccagggaa ggggctggag tgggttgga ggatcaggtc
 caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
 tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
 agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
 cttcggcaat tcttacgtgt cttgggtttgc ttattgggga caggggacac
 tgggtgactgt gtcttcaggga ggatgtggcg gtggagaagt ggccgcactg
 gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
 ggtcgcagcc ctggagaaag gcggcgggga caaaactcac acatgcccac
 cgtgcccagc acctgaagcc gcgggggggac cgtcagtctt cctcttcccc
 ccaaaaccca aggacaccct catgatctcc cggacccttg aggtcacatg
 cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag
 cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca
 ggactggctg aatggcaagg agtacaagt caaggtctcc aacaaagccc
 tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
 gaaccacagg tgtacaccct gcccccatcc cgggaggaga tgaccaagaa
 ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg

ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
 cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac
 cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgctccgtga
 tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
 ccgggtaaa

[00314] The amino acid sequence of the second polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 2 is (SEQ ID NO:222):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
 WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTITA DKSTSTAYME
 LSSLRSEDTA VYYCARIYGN NVYFDVWGQG TTVTVSSGGC GGGKVAALKE
 KVAALKEKVA ALKEKVAALK E

[00315] In SEQ ID NO:222, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (SEQ ID NO:186), residues 238-243 correspond to the linker GGC GGG (SEQ ID NO:34), and residues 244-271 are a K-coil Domain (SEQ ID NO:40).

[00316] A polynucleotide that encodes SEQ ID NO:222 is (SEQ ID NO:223):

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
 tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
 acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
 ggggggtacaa acaaaagggc tccttgacc cctgcacggc tttctggaag
 tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
 acgaagccga ttactattgt gctctgtggc atagcaatct gtgggtgttc
 ggggggtggca caaaactgac tgtgctggga ggggggtggat ccggcggagg
 tggacaggtc cagctgggtc agagcggggc cgaagtcaaa aaacccgagg
 caagcgtgaa ggtctcctgc aaagcatcag gctatacatt tacaggcagc
 tggatgaact ggggtgaggca ggctccagga cagggactgg agtggatcgg
 gcgcattctac cctggagacg gcgaaactaa ctataatgga aagttcaaa
 accgagtgac catcacagcc gataagtcta ctagtaccgc ctacatggag
 ctgagctccc tgcggtctga agataccgcc gtctactatt gcgctagaat
 ttacggaaac aatgtctatt ttgacgtgtg ggggcaggga acaactgtga
 ctgtctcctc cggaggatgt ggcgggtggaa aagtggccgc actgaaggag
 aaagttgctg ctttgaaaga gaaggctcgc gcacttaagg aaaaggctgc
 agccctgaaa gag

[00317] The amino acid sequence of the third polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 2 is (**SEQ ID NO:224**):

```
KVQLQQSGAE LVKPGASVKL SCKASGYTFT EYILHWVKQK SGQGLEWIGW
FYPGNNNIKY NEKFKDKATL TADKSSSTVY MELSRLTSED SAVYFCARHE
QGPGYFDYWG QGTTLTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFP AVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHNRYTQ KSLSLSPGK
```

[00318] In **SEQ ID NO:224**, amino acid residues 1-119 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (**SEQ ID NO:18**), residues 120-217 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 218-232 correspond to a linker (**SEQ ID NO:209**), and residues 233-449 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00319] A polynucleotide that encodes **SEQ ID NO:224** is (**SEQ ID NO:225**):

```
aaggtccagc tgcagcagtc tggagctgaa ctggtgaaac ccggggcattc
agtgaagctg tcctgcaagg cttctgggta caccttcact gagtatattt
tacactgggt aaagcagaag tctggacagg gtcttgagtg gattgggtgg
ttttatcctg gaaataataa tataaagtac aatgagaaat tcaaggacaa
ggccacactg actgcggaca aatcctccag cacagtctat atggaactta
gtagattgac atctgaagac tctgcggtct atttctgtgc aagacacgaa
caaggaccag gttactttga ctactggggc caaggcacca ctctcacagt
ctcctccgcc tccaccaagg gcccatcggt cttccccctg gcaccctcct
ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac
tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg ccctgaccag
cggcgtgcac accttccccg ctgtcctaca gtccctcagga ctctactccc
tcagcagcgt ggtgaccgtg ccctccagca gcttggggcac ccagacctac
atctgcaacg tgaatcacia gccagcaac accaagggtg acaagagagt
tgagcccaaa tcttgtagaca aaactcacac atgccaccg tgcccagcac
ctgaagccgc ggggggaccg tcagtcttcc tcttcccccc aaaacccaag
gacaccctca tgatctcccg gaccctgag gtcacatgcg tgggtggtgga
cgtgagccac gaagaccctg aggtcaagtt caactggtac gtggacggcg
tgagagtgca taatgccaag acaaagccgc gggaggagca gtacaacagc
acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa
tggaaggag tacaagtgca aggtctccaa caaagccctc ccagcccca
tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg
tacaccctgc ccccatcccc ggaggagatg accaagaacc aggtcagcct
gagttgcgca gtcaaaggct tctatccag cgacatcgcc gtggagtggg
agagcaatgg gcagccggag aacaactaca agaccacgcc tcccggtgctg
gactccgacg gctccttctt cctcgtcagc aagctcaccg tggacaagag
```

caggtggcag caggggaacg tcttctcatg ctccgtgatg catgaggctc
 tgcacaaccg ctacacgcag aagagcctct ccctgtctcc gggtaaa

[00320] The amino acid sequence of the fourth polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 2 is (**SEQ ID NO:226**):

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
 ASTRHTGVDP RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
 GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
 DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
 LSSPVTKSFN RGEC

[00321] In **SEQ ID NO:226**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (**SEQ ID NO:13**), and residues 108-214 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00322] A polynucleotide that encodes **SEQ ID NO:226** is (**SEQ ID NO:227**):

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
 cagggtcagc atcacctgca aggccagtcg ggatgtgaat actgctgtag
 cctgggtatca acaaaaacca gggcaatctc ctaaactact gatttactgg
 gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
 tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
 cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
 ggcaccaagc tggaaatcaa acgtacgggtg gctgcaccat cggctcttcac
 cttcccgcga tctgatgagc agttgaaatc tggaactgcc tctgtttgtgt
 gctgtctgaa taacttctat cccagagagg ccaaagtaca gtggaagggtg
 gataacgccc tccaatcggg taactcccag gagagtgtca cagagcagga
 cagcaaggac agcacctaca gcctcagcag caccctgacg ctgagcaaaag
 cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
 ctgagctcgc ccgtcacaaa gagcttcaac aggggagaggt gt

H. EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1

[00323] A further exemplary Tri-Specific Binding Molecule composed of four polypeptide chains was constructed. The Tri-Specific Binding Molecule comprises the VL and VH domains of EphA2 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of DR5 mAb 1, and was accordingly designated “EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:228**):

DIQMTQTTSS LSASLGDRIT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
 TSRLHSGVPS RFTGSGSGTD YSLTISNLEQ EDIATYFCQQ GYTLYTFGGG
 TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
 VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
 SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL

EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
 PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN QOPENNYKTT
 PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
 PGK

[00324] In **SEQ ID NO:228**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 1 (**SEQ ID NO:153**), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ ID NO:112**), residues 240-245 correspond to the GGCGGG linker (**SEQ ID NO:34**), residues 246-273 correspond to an E-coil Domain (**SEQ ID NO:39**), residues 274-276 are the linker GGG, residues 277-286 are the linker DKTHTCPPCP (**SEQ ID NO:48**), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**).

[00325] A polynucleotide that encodes **SEQ ID NO:228** is (**SEQ ID NO:229**):

gatataccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga
 cagaatcacc atcagttgca gggcaagtca ggacattagc aattatttaa
 actggtatca gcagaaacca gatggaactg ttaaactcct gatctactac
 acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc
 tggaacagat tattctctca ccattagcaa cctggagcaa gaagatatgt
 ccacttactt ttgccaacag ggttatacgc tgtacacggt cggagggggg
 accaagctgg aaataaaaagg tggaggatcc ggcggcggag gcgaggtgca
 gctggtggag tctgggggag gcttggtcca gcctggaggg tccctgagac
 tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
 gtccgccagg ctccagggaa ggggctggag tgggttgga ggatcaggtc
 caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
 tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
 agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
 cttcggcaat tcttacgtgt cttggtttgc ttattgggga caggggacac
 tgggtgactgt gtcttccgga ggatgtggcg gtggagaagt ggccgcactg
 gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
 ggtcgcagcc ctggagaaaag gcggcgggga caaaactcac acatgcccac
 cgtgcccagc acctgaagcc gcggggggac cgtcagtcct cctcttcccc
 ccaaaaccca aggacaccct catgatctcc cggacccttg aggtcacatg
 cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag
 cagtacaaca gcacgtaccg tgtggtcagc gtccctaccg tcctgcacca
 ggactggctg aatggcaagg agtacaagt caaggtctcc aacaaagccc
 tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
 gaaccacagg tgtacaccct gcccccatcc cgggaggaga tgaccaagaa

```

ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg
ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac
cgtggacaag agcagggtggc agcaggggaa cgtcttctca tgctccgtga
tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaa

```

[00326] The amino acid sequence of the second polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (SEQ ID NO:230):

```

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLKESGPGLV APSQSLSITC TVSGFSLSRV
SVHWVRQPPG KGLEWLGMIW GGGSTDYNSA LKSRLSISKD NSKSQVFLKM
NSLQTDDETAM YYCARKHGNY YTM DYWGQGT SVTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE

```

[00327] In SEQ ID NO:230, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 1 (SEQ ID NO:158), residues 237-242 correspond to the linker GGCGGG (SEQ ID NO:34), and residues 243-270 are a K-coil Domain (SEQ ID NO:40).

[00328] A polynucleotide that encodes SEQ ID NO:230 is (SEQ ID NO:231):

```

caggctgtgg tgactcagga gccttcaactg accgtgtccc caggcggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgac
gggggtacaa acaaaagggc tccctggacc cctgcacggc tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcggagg
tggaacaggtg cagctgaagg agtcaggacc tggcctgggt gcaccctcac
agagcctgtc catcacatgc actgtctctg ggttctcatt atccagatat
agtgtacact gggttcgcca gctccagga aagggtctgg agtggctggg
aatgatattg ggtggtggaa gcacagacta taattcagct ctcaaattcca
gactgagtat cagcaaggac aactccaaga gccaaagttt cttaaaaatg
aacagtctgc aaactgatga cacagccatg tactactgtg ccagaaaaca
tggtactac tatactatgg actactgggg tcaaggaaac tcagtcaccg
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag

```

[00329] The amino acid sequence of the third polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (SEQ ID NO:232):

```

EVKFLES GGG LVQPGGSLKL SCVASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTTRA
YYGNPAWFAY WGQGTLLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSL SCAVKGFYPSD IAVEWESNGQ PENNYKTTTP
VLDS DGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNRY TQKSLSLSPG
K

```

[00330] In SEQ ID NO:232, amino acid residues 1-121 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8), residues 122-219 correspond to a modified CH1 Domain (SEQ ID NO:208), residues 220-234 correspond to a linker (SEQ ID NO:209), and residues 235-451 correspond to the “hole-bearing” CH2-CH3 Domain (SEQ ID NO:53).

[00331] A polynucleotide that encodes SEQ ID NO:232 is (SEQ ID NO:233):

```

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tcctgtgtag cctcaggatt cgatttttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggagaa
attaatccag atagcaatac gataaactat acgccatctc taaaggataa
attcatcatc tccagagaca acgcaaaaaa tacgctgtat ctgcaaatga
ccaaagtgag atctgaggac acagcccttt attattgtac aagaagggcc
tactatggta acccggcctg gtttgcttac tggggccaag ggactctggt
cactgtctct tccgcctcca ccaagggcc atcggctctc cccctggcac
cctcctccaa gagcacctct gggggcacag cggccctggg ctgcctggtc
aaggactact tccccgaacc ggtgacggtg tcgtggaact caggcgccct
gaccagcggc gtgcacacct tcccggtgtg cctacagtcc tcaggactct
actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa
gagagttgag cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc
cagcacctga agccgcgggg ggaccgtcag tcttctctct cccccaaaa
cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgcgtggt
ggtggacgtg agccacgaag accctgaggt caagttcaac tggtagctgg
acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg
gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa gccctcccag
cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
caggtgtaca ccctgcccc atcccgaggag gagatgacca agaaccaggt
cagcctgagt tgcgcagtca aaggcttcta tcccagcgac atcgccgtgg
agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc
gtgctggact ccgacggctc cttcttctc gtcagcaagc tcaccgtgga

```

caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg
aggctctgca caaccgctac acgcagaaga gcctctccct gtctccgggt
aaa

[00332] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:234**):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLs STLTLKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00333] In **SEQ ID NO:234**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), and residues 112-218 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00334] A polynucleotide that encodes **SEQ ID NO:234** is (**SEQ ID NO:235**):

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcaactgtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaacgtacgg tggctgcacc
atcggctctc atcttcccgc catctgatga gcagttgaaa tctggaactg
cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta
cagtggaaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt
cacagagcag gacagcaagg acagcaccta cagcctcagc agcaccctga
cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga
gtgt

I. EphA2 mAb 2 x CD3 mAb 2 x DR5 mAb 1

[00335] A further exemplary Tri-Specific Binding Molecule composed of four polypeptide chains was constructed. The Tri-Specific Binding Molecule comprises the VL and VH domains of EphA2 mAb 2, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of DR5 mAb 1, and was accordingly designated “EphA2 mAb 2 x CD3 mAb 2 x DR5 mAb 1.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:236**):

DVVMQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
TFGSGTKLEI KGGGSGGGGE VQLVESGGGL VQPGGSLRLS CAASGFTFST

```

YAMNWVRQAP GKGLEWVGRI RSKYNNYATY YADSVKGRFT ISRDDSKNSL
YLQMNSLKTE DTAVYYCVRH GNFGNSYVSW FAYWGQGTLV TVSSGGCGGG
EVAALEKEVA ALEKEVAALE KEVAALEKGG GDKTHTCPPC PAPEAAGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSREEMT KNQVSLWCLV KGFYPSDIAV EWESNGQPEN
NYKTTTPPVL SDGSFFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK
SLSLSPGK

```

[00336] In **SEQ ID NO:236**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 2 (**SEQ ID NO:163**), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 120-244 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ ID NO:112**), residues 245-250 correspond to the GGCGGG linker (**SEQ ID NO:34**), residues 251-278 correspond to an E-coil Domain (**SEQ ID NO:39**), residues 279-281 are the linker GGG, residues 282-291 are the linker DKTHTCPPCP (**SEQ ID NO:48**), and residues 292-508 are the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**).

[00337] A polynucleotide that encodes **SEQ ID NO:236** is (**SEQ ID NO:237**):

```

gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttgagaga
tcaagcctcc atctcttgca gatctagtca gagccttgta cacagtagtg
gaaacaccta ttacattgg tacctgcaga agccaggcca gtctccaaag
ctcctgatct acaaagtttc caaccgatct tctgggggtcc cagacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgttccc
acgttcggct cggggacaaa gttggaata aaaggtggag gatccggcgg
cggaggcgag gtgcagctgg tggagtctgg gggaggcttg gtccagcctg
gagggtccct gagactctcc tgtgcagcct ctggattcac cttcagcaca
tacgctatga attgggtccg ccaggctcca gggaaggggc tggagtgggt
tggaaggatc aggtccaagt acaacaatta tgcaacctac tatgccgact
ctgtgaaggg tagattcacc atctcaagag atgattcaaa gaactcactg
tatctgcaaa tgaacagcct gaaaaccgag gacacggccg tgtattactg
tgtgagacac ggtaacttcg gcaattctta cgtgtcttgg tttgcttatt
ggggacaggg gacactggtg actgtgtctt ccggaggatg tggcgggtgga
gaagtggccg cactggagaa agaggttgct gctttggaga aggaggtcgc
tgcacttgaa aaggaggtcg cagccctgga gaaaggcggc ggggacaaaa
ctcacacatg cccaccgtgc ccagcacctg aagccgcggg gggaccgtca
gtcttcctct tcccccaaaa acccaaggac accctcatga tctcccgac
ccctgaggtc acatgcgtgg tgggtggacgt gagccacgaa gaccctgagg
tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct
caccgtcctg caccaggact ggctgaatgg caaggagtac aagtgcgaagg

```

```

tctccaacaa agccctccca gcccccatcg agaaaaccat ctccaaagcc
aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga
ggagatgacc aagaaccagg tcagcctgtg gtgcctgggtc aaaggcttct
atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct
ctacagcaag ctcaccgtgg acaagagcag gtggcagcag gggaacgtct
tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag
agcctctccc tgtctccggg taaa

```

[00338] The amino acid sequence of the second polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:238**):

```

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQI QLVQSGPELK KPGETVKISC KASGFTFTNY
GMNWVKQAPG KGLKWMGWIN TYIGEPTYAD DFKGRFVFSL ETSASTAYLQ
INNLKNEDMA TYFCARELGP YYFDYWQGT TLTVSSGGCG GKKVAALKEK
VAALKEKVAA LKEKVAALKE

```

[00339] In **SEQ ID NO:238**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 2 (**SEQ ID NO:167**), residues 237-242 correspond to the linker GGCGGG (**SEQ ID NO:34**), and residues 243-270 are a K-coil Domain (**SEQ ID NO:40**).

[00340] A polynucleotide that encodes **SEQ ID NO:238** is (**SEQ ID NO:239**):

```

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcgggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggg atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg
tgacagatc cagttggtgc agtctggacc tgagctgaag aagcctggag
agacagtcaa gatctcctgc aaggcttctg ggtttacctt cacaaactat
ggaatgaact gggatgaagca ggctccagga aagggtttta agtggtatggg
ctggataaac acctatattg gagagccgac atatgctgat gacttcaagg
gacggtttgt cttctctttg gaaacctctg ccagcactgc ctatttgag
atcaacaacc tcaaaaatga ggacatggcc acatatcttct gtgcaagaga
actgggacca tactactttg actactgggg ccaaggcacc actctcacag
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag

```

[00341] The amino acid sequence of the third polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x DR5 mAb 1 is (SEQ ID NO:240):

```

EVKFLES GGG LVQPGGSLKL SCVASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTTRA
YYGNPAWFAY WGQGTLLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSL SCAVKGFYPSD IAVEWESNGQ PENNYKTTTP
VLDS DGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNRY TQKSLSLSPG
K

```

[00342] In SEQ ID NO:240, amino acid residues 1-121 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8), residues 122-219 correspond to a modified CH1 Domain (SEQ ID NO:208), residues 220-234 correspond to a linker (SEQ ID NO:209), and residues 235-451 correspond to the “hole-bearing” CH2-CH3 Domain (SEQ ID NO:53).

[00343] A polynucleotide that encodes SEQ ID NO:240 is (SEQ ID NO:241):

```

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tcctgtgtag cctcaggatt cgatttttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggagaa
attaatccag atagcaatac gataaactat acgccatctc taaaggataa
attcatcatc tccagagaca acgcaaaaaa tacgctgtat ctgcaaatga
ccaaagtgag atctgaggac acagcccttt attattgtac aagaagggcc
tactatggta acccggcctg gtttgcttac tggggccaag ggactctggt
cactgtctct tccgcctcca ccaagggcc atcggctctc cccctggcac
cctcctccaa gagcacctct gggggcacag cggccctggg ctgcctggtc
aaggactact tccccgaacc ggtgacggtg tcgtggaact caggcgccct
gaccagcggc gtgcacacct tcccggtgtg cctacagtcc tcaggactct
actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa
gagagttgag cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc
cagcacctga agccgcgggg ggaccgtcag tcttctctct cccccaaaaa
cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgcgtggt
ggtggacgtg agccacgaag accctgaggt caagttcaac tggtagctgg
acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg
gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa gccctcccag
cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
caggtgtaca ccctgcccc atcccgaggag gagatgacca agaaccaggt
cagcctgagt tgcgcagtca aaggcttcta tcccagcgac atcgccgtgg
agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc
gtgctggact ccgacggctc cttcttctc gtcagcaagc tcaccgtgga

```

caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg
aggctctgca caaccgctac acgcagaaga gcctctccct gtctccgggt
aaa

[00344] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:242**):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLs STLTLKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00345] In **SEQ ID NO:242**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), and residues 112-218 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00346] A polynucleotide that encodes **SEQ ID NO:242** is (**SEQ ID NO:243**):

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcaactgtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaacgtacgg tggctgcacc
atcggctctc atcttcccgc catctgatga gcagttgaaa tctggaactg
cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta
cagtggaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt
cacagagcag gacagcaagg acagcaccta cagcctcagc agcaccctga
cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga
gtgt

J. EphA2 mAb 3 x CD3 mAb 2 x DR5 mAb 1

[00347] A further exemplary Tri-Specific Binding Molecule composed of four polypeptide chains was constructed. The Tri-Specific Binding Molecule comprises the VL and VH domains of EphA2 mAb 3, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of DR5 mAb 1, and was accordingly designated “**EphA2 mAb 3 x CD3 mAb 2 x DR5 mAb 1**.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:244**):

DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAWAWYQQKP GQSPKLLIFW
ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN

```

WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISR DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGT LVT VSS GCGGGGEVAA
LEKEVA ALEK EVA ALEKEVA ALEKGGGDKT HTCP PCPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

```

[00348] In **SEQ ID NO:244**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 3 (**SEQ ID NO:172**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-240 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ ID NO:112**), residues 241-246 correspond to the GCGGGG linker (**SEQ ID NO:34**), residues 247-274 correspond to an E-coil Domain (**SEQ ID NO:39**), residues 275-277 are the linker GGG, residues 278-287 are the linker DKHTTCPPCP (**SEQ ID NO:48**), and residues 288-504 are the “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:52**).

[00349] A polynucleotide that encodes **SEQ ID NO:244** is (**SEQ ID NO:245**):

```

gacattgtgc tgacccagtc tcacagatcc atgtccacat cagtaggaga
caggggtcaac atcacctgca aggcagtcga ggatgtgact actgctgtag
cctgggtatca acaaaaacca gggcaatctc ctaaattact gattttctgg
gcatccaccc ggcacgctgg agtccctgat cgcttcacag gcagtggatc
tgggacagat ttactctca ccatcagcag tgtgcaggct ggagacctgg
cactttatta ctgtcaacaa cattatagca caccgtacac attcggaggg
gggaccaagc tggaaataaa aggtggagga tccggcggcg gaggcgagggt
gcagctggtg gagtctgggg gaggcttggg ccagcctgga ggggtccctga
gactctcctg tgcagcctct ggattcacct tcagcacata cgctatgaat
tggggtccgcc aggtctccagg gaaggggctg gagtggggtg gaaggatcag
gtccaagtac aacaattatg caacctacta tgccgactct gtgaagggtg
gattcaccat ctcaagagat gattcaaaga actcactgta tctgcaaagt
aacagcctga aaaccgagga cacggccgtg tattactgtg tgagacacgg
taacttcggc aattcttacg tgtcttggtt tgcttattgg ggacagggga
cactgggtgac tgtgtcttcc ggaggatgtg gcggtggaga agtggccgca
ctggagaaag aggttgctgc tttggagaag gaggtcgctg cacttgaaaa
ggaggtcgca gccctggaga aaggcggcgg ggacaaaact cacacatgcc
caccgtgccc agcacctgaa gccgcggggg gaccgtcagt cttcctcttc
ccccaaaac ccaaggacac cctcatgata tcccggaacc ctgagggtcac
atgcgtggtg gtggacgtga gccacgaaga ccctgagggtc aagttcaact
ggtacgtgga cggcgtggag gtgcataatg ccaagacaaa gccgcgggag
gagcagtaca acagcacgta ccgtgtggtc agcgtcctca ccgtcctgca
ccaggactgg ctgaatggca aggagtacaa gtgcaagggtc tccaacaaag

```

```

ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc
cgagaaccac aggtgtacac cctgccccca tcccgggagg agatgaccaa
gaaccaggtc agcctgtggt gcctggtcaa aggcttctat cccagcgaca
tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc
acgcctcccg tgctggactc cgacggctcc ttcttcctct acagcaagct
caccgtggac aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg
tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg
tctccgggta aa

```

[00350] The amino acid sequence of the second polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x DR5 mAb 1 is (SEQ ID NO:246):

```

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGEV QLVESGGGSV KPGGSLKLSC AASGFTFTDH
YMYWVRQTPE KRLEWVATIS DGSFSTSYPD SVKGRFTISR DIAKNNLYLQ
MSSLKSEDTA MYYCTRDESD RFPFYWGQGT LVTVSSGGCG GKKVAALKEK
VAALKEKVAA LKEKVAALKE

```

[00351] In SEQ ID NO:246, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 3 (SEQ ID NO:177), residues 237-242 correspond to the linker GGCGGG (SEQ ID NO:34), and residues 243-270 are a K-coil Domain (SEQ ID NO:40).

[00352] A polynucleotide that encodes SEQ ID NO:246 is (SEQ ID NO:247):

```

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg
tgagaaagtg cagctggtgg agtctggggg aggctcagtg aagcctggag
ggtccctgaa actctcctgt gcagcctctg gattcacttt cactgaccat
tacatgtatt gggttcgcca gactccggaa aagaggctgg agtgggtcgc
aaccattagt gatggcggta gtttcacctc ctatccagac agtgtgaagg
ggcgattcac catctccaga gacattgcca agaacaacct gtacctcaa
atgagcagtc tgaagtctga ggacacagcc atgtattact gtacaagaga
tgagagcgat aggccgtttc cttactgggg ccaagggact ctggtcactg
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag

```

[00353] The amino acid sequence of the third polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x DR5 mAb 1 is (SEQ ID NO:248):

```

EVKFLES GGG LVQPGGSLKL SCVASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTTRA
YYGNPAWFAY WGQGTLLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSL SCAVKGFYPSD IAVEWESNGQ PENNYKTTTP
VLDS DGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNRY TQKSLSLSPG
K

```

[00354] In SEQ ID NO:248, amino acid residues 1-121 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8), residues 122-219 correspond to a modified CH1 Domain (SEQ ID NO:208), residues 220-234 correspond to a linker (SEQ ID NO:209), and residues 235-451 correspond to the “hole-bearing” CH2-CH3 Domain (SEQ ID NO:53).

[00355] A polynucleotide that encodes SEQ ID NO:248 is (SEQ ID NO:249):

```

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tcctgtgtag cctcaggatt cgatttttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggagaa
attaatccag atagcaatac gataaactat acgccatctc taaaggataa
attcatcatc tccagagaca acgcaaaaaa tacgctgtat ctgcaaatga
ccaaagtgag atctgaggac acagcccttt attattgtac aagaagggcc
tactatggta acccggcctg gtttgcttac tggggccaag ggactctggt
cactgtctct tccgcctcca ccaagggcc atcggctctc ccctggcac
cctcctccaa gagcacctct gggggcacag cggccctggg ctgcctggtc
aaggactact tccccgaacc ggtgacggtg tcgtggaact caggcgccct
gaccagcggc gtgcacacct tcccggtgtg cctacagtcc tcaggactct
actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa
gagagttgag cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc
cagcacctga agccgcgggg ggaccgtcag tcttctctct cccccaaaa
cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgcgtggt
ggtggacgtg agccacgaag accctgaggt caagttcaac tggtagctgg
acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg
gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa gccctcccag
cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
caggtgtaca ccctgcccc atcccgaggag gagatgacca agaaccaggt
cagcctgagt tgcgcagtca aaggcttcta tcccagcgac atcgccgtgg
agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc
gtgctggact ccgacggctc cttcttctc gtcagcaagc tcaccgtgga

```

caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg
aggctctgca caaccgctac acgcagaaga gcctctccct gtctccgggt
aaa

[00356] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x DR5 mAb 1 is (**SEQ ID NO:250**):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLs STLTLKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00357] In **SEQ ID NO:250**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), and residues 112-218 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00358] A polynucleotide that encodes **SEQ ID NO:250** is (**SEQ ID NO:251**):

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcaactgtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaacgtacgg tggctgcacc
atcggctctc atcttcccgc catctgatga gcagttgaaa tctggaactg
cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta
cagtggaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt
cacagagcag gacagcaagg acagcaccta cagcctcagc agcaccctga
cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc
accatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga
gtgt

[00359] Although the exemplary Tri-Specific Binding Molecules described above comprise three Light Chain (VL) CDRs and three Heavy Chain (VH) CDRs for each binding domain, it will be recognized that the invention also includes Tri-Specific Binding Molecules that possess:

- (1) at least one of the CDRs of the VL Domain of any such binding domain;
- (2) at least two of the CDRs of the VL Domain of any such binding domain;
- (3) the three CDRs of the VL Domain of any such binding domain;

- (4) at least one of the CDRs of the VH Domain of any such binding domain;
- (5) at least two of the CDRs of the VH Domain of any such binding domain;
- (6) the three CDRs of the VH Domain of any such binding domain;
- (7) at least one of the CDRs of the VL Domain of any such binding domain and at least one of the CDRs of the VH Domain of that binding domain;
- (8) at least two of the CDRs of the VL Domain of any such binding domain and at least two of the CDRs of the VH Domain of that binding domain;
- (9) the three CDRs of the VL Domain of any such binding domain and the three CDRs of the VH Domain of that binding domain;
- (10) the VL Domain of any such binding domain;
- (11) the VH Domain of any such binding domain; or
- (12) the VL and VH Domains of any such binding domain.

K. gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 1

[00360] A Tri-Specific Binding Molecule composed of four polypeptide chains was constructed that comprises the VL and VH domains of gpA33 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of EphA2 mAb 1. The Tri-Specific Binding Molecule was accordingly designated “**gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 1.**” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:252**):

```

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK

```

[00361] In SEQ ID NO:252, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (SEQ ID NO:181), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 240-245 correspond to the GGCGGG linker (SEQ ID NO:34), residues 246-273 correspond to an E-coil Domain (SEQ ID NO:39), residues 274-276 are the linker GGG, residues 277-286 are the linker DKHTTCPPCP (SEQ ID NO:48), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00362] A polynucleotide that encodes SEQ ID NO:252 is (SEQ ID NO:253):

```

gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggtc ctcaatcagc ttcatgtact
ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaaggg tggaggatcc ggcggcggag gcgagggtgca
gctggtggag tctgggggag gcttggtcca gcctggaggg tccctgagac
tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
gtccgccagg ctccaggga ggggctggag tgggttgga ggatcaggtc
caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
cttcggcaat tcttacgtgt cttgggtttg ttattgggga caggggacac
tggtgactgt gtcttccgga ggatgtggcg gtggagaagt ggccgcaactg
gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
ggtcgcagcc ctggagaaa ggcggcggga caaaactcac acatgccac
cgtgcccagc acctgaagcc gcggggggac cgtcagtctt cctcttcccc
ccaaaaccca aggacaccct catgatctcc cggaccctg aggtcacatg
cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
acgtggacgg cgtggagggt cataatgcca agacaaagcc gcgggaggag
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca
ggactggctg aatggcaagg agtacaagt caaggtctcc aacaaagccc
tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
gaaccacagg tgtacaccct gcccctatcc cgggaggaga tgaccaagaa
ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg
ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac
cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgctccgtga
tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaaa

```

[00363] The amino acid sequence of the second polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 1 is (**SEQ ID NO:254**):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARIYGN NVYFDVWGQG TTVTVSSGGC GGGKVAALKE
KVAALKEKVA ALKEKVAALK E
```

[00364] In **SEQ ID NO:254**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (**SEQ ID NO:186**), residues 238-243 correspond to the linker GGC GGG (**SEQ ID NO:34**), and residues 244-271 are a K-coil Domain (**SEQ ID NO:40**).

[00365] A polynucleotide that encodes **SEQ ID NO:254** is (**SEQ ID NO:255**):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcgagg
tggacaggtc cagctggtcc agagcggggc cgaagtcaaa aaacccgagg
caagcgtgaa ggtctcctgc aaagcatcag gctatacatt tacaggcagc
tggatgaact ggggtgaggca ggctccagga cagggactgg agtggatcgg
gcgcatctac cctggagacg gcgaaactaa ctataatgga aagttcaaa
accgagtgac catcacagcc gataagtcta ctagtaccgc ctacatggag
ctgagctccc tgcggtctga agataccgcc gtctactatt gcgctagaat
ttacggaaac aatgtctatt ttgacgtgtg ggggcaggga acaactgtga
ctgtctcctc cggaggatgt ggcggtggaa aagtggccgc actgaaggag
aaagttgctg ctttgaaaga gaaggtcgcc gcacttaagg aaaaggtcgc
agccctgaaa gag
```

[00366] The amino acid sequence of the third polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 1 is (**SEQ ID NO:256**):

```
QVQLKESGPG LVAPSQSLSI TCTVSGFSLs RYSVHWVRQP PGKGLEWLGM
IWGGGSTDYN SALKSRLSIS KDNSKSQVFL KMNSLQTDDT AMYYCARKHG
NYYTMDYWGQ GTSVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPEAAGGPS VFLFPPKPKD
```

TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
 TLPPSREEMT KNQVSLSCAV KGFYPSDIAV EWESNGQPEN NYKTTFPVLD
 SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH EALHNRYTQK SLSLSPGK

[00367] In **SEQ ID NO:256**, amino acid residues 1-118 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 1 (**SEQ ID NO:158**), residues 119-216 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 217-231 correspond to a linker (**SEQ ID NO:209**), and residues 232-448 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00368] A polynucleotide that encodes **SEQ ID NO:256** is (**SEQ ID NO:257**):

caggtgcagc tgaaggagtc aggacctggc ctggtggcac cctcacagag
 cctgtccatc acatgcactg tctctgggtt ctcatatatt agatatagtg
 tacactgggt tcgccagcct ccaggaaagg gtctggagtg gctgggaatg
 atatgggggt gtggaagcac agactataat tcagctctca aatccagact
 gagtatcagc aaggacaact ccaagagcca agttttctta aaaatgaaca
 gtctgcaaac tgatgacaca gccatgtact actgtgccag aaaacatggg
 aactactata ctatggacta ctgggggtcaa ggaacctcag tcaccgtctc
 ctccgcctcc accaagggcc catcggtctt ccccttgcca ccctcctcca
 agagcacctc tgggggcaca gcggccctgg gctgcctggg caaggactac
 ttccccgaac cgggtgacggg gtcgtggaac tcaggcgccc tgaccagcgg
 cgtgcacacc ttcccggctg tcttacagtc ctacaggact tactccctca
 gcagcgtggg gaccgtgccc tccagcagct tgggcaccca gacctacatc
 tgcaacgtga atcacaagcc cagcaacacc aaggtggaca agagagttga
 gcccaaattc tgtgacaaaa ctacacacatg cccaccgtgc ccagcacctg
 aagccgcggg gggaccgtca gtcttcctct tcccccaaa acccaaggac
 accctcatga tctcccgga ccttgagggtc acatgcgtgg tgggtggacgt
 gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg
 aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg
 taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg
 caaggagtac aagtgcgaag tctccaacaa agccctccca gccccatcg
 agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac
 accctgcccc catcccggga ggagatgacc aagaaccagg tcagcctgag
 ttgctgcagtc aaaggcttct atcccagcga catcgccgtg gagggggaga
 gcaatgggca gccgggagaa aactacaaga ccacgcctcc cgtgctggac
 tccgacggct ccttcttcct cgtcagcaag ctaccctggg acaagagcag
 gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc
 acaaccgcta cacgcagaag agcctctccc tgtctccggg taaa

[00369] The amino acid sequence of the fourth polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 1 is (**SEQ ID NO:258**):

DIQMTQTSS LSASLGDRIT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
 TSRHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GYTLTYFGGG
 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNHFYP REAKVQWKVD

NALQSGNSQE SVTEQDSKDS TYSLSTLTL SKADYEKHKV YACEVTHQGL
SSPVTKSFNR GEC

[00370] In **SEQ ID NO:258**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 1 (**SEQ ID NO:153**), and residues 107-213 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00371] A polynucleotide that encodes **SEQ ID NO:258** is (**SEQ ID NO:259**):

```
gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga
cagaatcacc atcagttgca gggcaagtca ggacattagc aattatttaa
actggtatca gcagaaacca gatggaactg ttaaactcct gatctactac
acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc
tggaacagat tattctctca ccattagcaa cctggagcaa gaagatattg
ccacttactt ttgccaacag gggtatacgc tgtacacgtt cggagggggg
accaagctgg aaataaaaacg tacggtggct gcaccatcgg tcttcatctt
cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc
tgctgaataa cttctatccc agagaggcca aagtacagtg gaaggtggat
aacgccctcc aatcggttaa ctcccaggag agtgtcacag agcaggacag
caaggacagc acctacagcc tcagcagcac cctgacgctg agcaaagcag
actacgagaa acacaaagtc tacgcctgcg aagtcacca tcagggcctg
agctcgcccg tcacaaagag cttcaacagg ggagagtgt
```

L. gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 2

[00372] A Tri-Specific Binding Molecule composed of four polypeptide chains was constructed that comprises the VL and VH domains of gpA33 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of EphA2 mAb 2. The Tri-Specific Binding Molecule was accordingly designated “**gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 2**.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:260**):

```
DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQW SSYPLTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

[00373] In SEQ ID NO:260, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (SEQ ID NO:181), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 240-245 correspond to the GGCGGG linker (SEQ ID NO:34), residues 246-273 correspond to an E-coil Domain (SEQ ID NO:39), residues 274-276 are the linker GGG, residues 277-286 are the linker DKHTTCPPCP (SEQ ID NO:48), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00374] A polynucleotide that encodes SEQ ID NO:260 is (SEQ ID NO:261):

```

gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggtc ctcaatcagc ttcatgtact
ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaaggg tggaggatcc ggcggcggag gcgagggtgca
gctgggtggag tctggggggag gcttggtcca gcctggaggg tccctgagac
tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
gtccgccagg ctccaggga ggggctggag tgggttgga ggatcaggtc
caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
cttcggcaat tcttacgtgt cttgggtttgc ttattgggga caggggacac
tggtgactgt gtcttccgga ggatgtggcg gtggagaagt ggccgcaactg
gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
ggtcgcagcc ctggagaaa ggcggcggga caaaactcac acatgccac
cgtgcccagc acctgaagcc gcggggggac cgtcagtctt cctcttcccc
ccaaaaccca aggacaccct catgatctcc cggaccctg aggtcacatg
cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
acgtggacgg cgtggagggt cataatgcca agacaaagcc gcgggaggag
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca
ggactggctg aatggcaagg agtacaagt caaggtctcc aacaaagccc
tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
gaaccacagg tgtacacct gcccccatcc cgggaggaga tgaccaagaa
ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg
ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac
cgtggacaag agcagggtgg agcaggggaa cgtcttctca tgctccgtga
tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaaa

```

[00375] The amino acid sequence of the second polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 2 is (SEQ ID NO:262):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARIYGN NVYFDVWGQG TTVTVSSGGC GGGKVAALKE
KVAALKEKVA ALKEKVAALK E
```

[00376] In SEQ ID NO:262, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (SEQ ID NO:186), residues 238-243 correspond to the linker GGC GGG (SEQ ID NO:34), and residues 244-271 are a K-coil Domain (SEQ ID NO:40).

[00377] A polynucleotide that encodes SEQ ID NO:262 is (SEQ ID NO:263):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcgggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcggagg
tggacaggtc cagctggtcc agagcggggc cgaagtcaaa aaaccgggag
caagcgtgaa ggtctcctgc aaagcatcag gctatacatt tacaggcagc
tggatgaact ggggtgaggca ggctccagga cagggactgg agtggatcgg
gcgcatctac cctggagacg gcgaaactaa ctataatgga aagttcaaaag
accgagtgac catcacagcc gataagtcta ctagtaccgc ctacatggag
ctgagctccc tgcggtctga agataccgcc gtctactatt gcgctagaat
ttacggaaac aatgtctatt ttgacgtgtg ggggcaggga acaactgtga
ctgtctcctc cggaggatgt ggcggtggaa aagtggccgc actgaaggag
aaagttgctg ctttgaaaga gaaggtcgcc gcacttaagg aaaaggtcgc
agccctgaaa gag
```

[00378] The amino acid sequence of the third polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 2 is (SEQ ID NO:264):

```
QIQLVQSGPE LKKPGETVKI SCKASGFTFT NYGMNWVKQA PGKGLKWMGW
INTYIGEPTY ADDFKGRFVF SLETSASTAY LQINNPKNED MATYFCAREL
GPYYFDYWGQ GTTLTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPEAAGGPS VFLFPPKPKD
```

TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
 TLPPSREEMT KNQVSLSCAV KGFYPSDIAV EWESNGQPEN NYKTTTPFVLD
 SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH EALHNRYTQK SLSLSPGK

[00379] In **SEQ ID NO:264**, amino acid residues 1-118 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 2 (**SEQ ID NO:167**), residues 119-216 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 217-231 correspond to a linker (**SEQ ID NO:209**), and residues 232-448 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00380] A polynucleotide that encodes **SEQ ID NO:264** is (**SEQ ID NO:265**):

cagatccagt tgggtgcagtc tggacctgag ctgaagaagc ctggagagac
 agtcaagatc tcctgcaagg cttctgggtt taccttcaca aactatggaa
 tgaactgggt gaagcaggct ccaggaaagg gtttaaagtg gatgggctgg
 ataaacacct atattggaga gccgacatat gctgatgact tcaagggacg
 gtttgtcttc tctttggaaa cctctgccag cactgcctat ttgcagatca
 acaacctcaa aaatgaggac atggccacat atttctgtgc aagagaactg
 ggaccatact actttgacta ctggggccaa ggcaccactc tcacagtctc
 ctccgcctcc accaagggcc catcggtctt ccccttgcca ccctcctcca
 agagcacctc tgggggcaca gcggccctgg gctgcctggt caaggactac
 ttccccgaac cggtgacggt gtcgtggaac tcaggcgccc tgaccagcgg
 cgtgcacacc ttcccggctg tcttacagtc ctcaggactc tactccctca
 gcagcgtggt gaccgtgccc tccagcagct tgggcaccca gacctacatc
 tgcaacgtga atcacaagcc cagcaacacc aaggtggaca agagagttga
 gcccaaattc tgtgacaaaa ctcacacatg cccaccgtgc ccagcacctg
 aagccgcggg gggaccgtca gtcttcctct tcccccaaa acccaaggac
 accctcatga tctcccgga ccttgaggtc acatgcgtgg tgggtggacgt
 gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg
 aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg
 taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg
 caaggagtac aagtgcaagg tctccaacaa agccctccca gccccatcg
 agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac
 accctgcccc catcccggga ggagatgacc aagaaccagg tcagcctgag
 ttgctgcagtc aaaggcttct atcccagcga catcgccgtg gagtgggaga
 gcaatgggca gccggagaa aactacaaga ccacgcctcc cgtgctggac
 tccgacggct ccttcttcct cgtcagcaag ctcaccgtgg acaagagcag
 gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc
 acaaccgcta cacgcagaag agcctctccc tgtctccggg taaa

[00381] The amino acid sequence of the fourth polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 2 is (**SEQ ID NO:266**):

DVVMTQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
 LLIIYKVS NFR SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
 TFGSGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV

QWKVDNALQS GNSQESVTEQ DSKDSTYSLs STLTLsKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00382] In **SEQ ID NO:266**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 1 (**SEQ ID NO:163**), and residues 112-218 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00383] A polynucleotide that encodes **SEQ ID NO:266** is (**SEQ ID NO:267**):

```
gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga
tcaagcctcc atctcttgca gatctagtca gagccttgta cacagtagtg
gaaacaccta ttacattgg tacctgcaga agccaggcca gtctccaaag
ctcctgatct acaaagtttc caaccgatth tctgggggtcc cagacaggtt
cagtggcagt ggatcaggga cagatttcac actcaagatc agcagagtgg
aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgttccc
acgttcggct cggggacaaa gttggaaata aaacgtacgg tggctgcacc
atcggctctc atcttcccgc catctgatga gcagttgaaa tctggaactg
cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta
cagtggaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt
cacagagcag gacagcaagg acagcaccta cagcctcagc agcaccctga
cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga
gtgt
```

M. gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 3

[00384] A Tri-Specific Binding Molecule composed of four polypeptide chains was constructed that comprises the VL and VH domains of gpA33 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of EphA2 mAb 3. The Tri-Specific Binding Molecule was accordingly designated “**gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 3**.” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:268**):

```
DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISsLEAE DAATYYCQQW SSYPLTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLsCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRsKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGnFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALeKE VAALEKEVAA LEKGGGDkTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLsLS
PGK
```

[00385] In SEQ ID NO:268, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (SEQ ID NO:181), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 240-245 correspond to the GGCGGG linker (SEQ ID NO:34), residues 246-273 correspond to an E-coil Domain (SEQ ID NO:39), residues 274-276 are the linker GGG, residues 277-286 are the linker DKHTTCPPCP (SEQ ID NO:48), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00386] A polynucleotide that encodes SEQ ID NO:268 is (SEQ ID NO:269):

```

gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggtc ctcaatcagc ttcatgtact
ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaaggg tggaggatcc ggcggcggag gcgagggtgca
gctgggtggag tctggggggg gcttggtcca gcctggaggg tccctgagac
tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
gtccgccagg ctccaggga ggggctggag tgggttgga ggatcaggtc
caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
cttcggcaat tcttacgtgt cttgggtttg ttattgggga caggggacac
tggtgactgt gtcttccgga ggatgtggcg gtggagaagt ggccgcaactg
gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
ggtcgcagcc ctggagaaa ggcggcggga caaaactcac acatgccac
cgtgcccagc acctgaagcc gcggggggac cgtcagtctt cctcttcccc
ccaaaaccca aggacaccct catgatctcc cggaccctg aggtcacatg
cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca
ggactggctg aatggcaagg agtacaagt caaggtctcc aacaaagccc
tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
gaaccacagg tgtacaccct gcccctatcc cgggaggaga tgaccaagaa
ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg
ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac
cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgctccgtga
tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaaa

```

[00387] The amino acid sequence of the second polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 3 is (SEQ ID NO:270):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARIYGN NVYFDVWGQG TTVTVSSGGC GGGKVAALKE
KVAALKEKVA ALKEKVAALK E
```

[00388] In SEQ ID NO:270, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (SEQ ID NO:186), residues 238-243 correspond to the linker GGC GGG (SEQ ID NO:34), and residues 244-271 are a K-coil Domain (SEQ ID NO:40).

[00389] A polynucleotide that encodes SEQ ID NO:270 is (SEQ ID NO:271):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcgggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaatg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcggagg
tggacaggtc cagctggtcc agagcggggc cgaagtcaaa aaaccgcggag
caagcgtgaa ggtctcctgc aaagcatcag gctatacatt tacaggcagc
tggatgaact ggggtgaggca ggctccagga cagggactgg agtggatcgg
gcgcactctac cctggagacg gcgaaactaa ctataatgga aagttcaaaag
accgagtgc catcacagcc gataagtcta ctagtaccgc ctacatggag
ctgagctccc tgcggtctga agataccgcc gtctactatt gcgctagaat
ttacggaaac aatgtctatt ttgacgtgtg ggggcaggga acaactgtga
ctgtctcctc cggaggatgt ggcggtggaa aagtggccgc actgaaggag
aaagttgctg ctttgaaaga gaaggtcgcc gcacttaagg aaaaggtcgc
agccctgaaa gag
```

[00390] The amino acid sequence of the third polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 3 is (SEQ ID NO:272):

```
EVQLVESGGG SVKPGGSLKL SCAASGFTFT DHYMYWVRQT PEKRLWVAT
ISDGSFSTSY PDSVKGRFTI SRDIKNNLY LQMSSLKSED TAMYCYTRDE
SDRPFYPYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPEAAGGPS VFLFPPKPKD
```

TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
 TLPPSREEMT KNQVSLSCAV KGFYPSDIAV EWESNGQPEN NYKTTFPVLD
 SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH EALHNRYTQK SLSLSPGK

[00391] In **SEQ ID NO:272**, amino acid residues 1-118 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 3 (**SEQ ID NO:177**), residues 119-216 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 217-231 correspond to a linker (**SEQ ID NO:209**), and residues 232-448 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00392] A polynucleotide that encodes **SEQ ID NO:272** is (**SEQ ID NO:273**):

gaagtgcagc tgggtggagtc tggggggaggc tcagtgaagc ctggaggggtc
 cctgaaactc tcctgtgcag cctctggatt cactttcact gaccattaca
 tgtattgggt tcgccagact ccggaagaaggc ggctggagtg ggctcgcaacc
 attagtgatg gcggtagttt cacctcctat ccagacagtg tgaaggggctg
 attcaccatc tccagagaca ttgccaaagaa caacctgtac ctccaaatga
 gcagtctgaa gtctgaggac acagccatgt attactgtac aagagatgag
 agcgataggc cgtttcctta ctggggccaa gggactcttg tcaactgtctc
 ctccgcctcc accaagggcc catcggtctt cccctctggc ccctcctcca
 agagcacctc tgggggcaca gcggccctgg gctgcctggc caaggactac
 ttccccgaac cgggtgacggc gtcgtggaac tcaggcgccc tgaccagcgg
 cgtgcacacc ttcccggctg tcttacagtc ctcaggactc tactccctca
 gcagcgtggt gaccgtgccc tccagcagct tgggcaccca gacctacatc
 tgcaacgtga atcacaagcc cagcaaacacc aaggtggaca agagagttga
 gcccaaattc tgtgacaaaa ctcacacatg cccaccgtgc ccagcacctg
 aagccgcggg gggaccgtca gtcttcctct tcccccaaa acccaaggac
 accctcatga tctcccgga ccttgaggct acatgcgtgg tgggtggacgt
 gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg
 aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg
 taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg
 caaggagtac aagtgcgaag tctccaacaa agccctccca gcccccatcg
 agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acagggtgtac
 accctgcccc catcccgga ggagatgacc aagaaccagg tcagcctgag
 ttgctgcagtc aaaggcttct atccagcga catcgccgtg gagggggaga
 gcaatgggca gccggagaa aactacaaga ccacgcctcc cgtgctggac
 tccgacggct ccttcttcct cgtcagcaag ctcaccgtgg acaagagcag
 gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc
 acaaccgcta cacgcagaag agcctctccc tgtctccggg taaa

[00393] The amino acid sequence of the fourth polypeptide chain of gpA33 mAb 1 x CD3 mAb 2 x EphA2 mAb 3 is (**SEQ ID NO:274**):

DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAVAWYQQKP GQSPKLLIFW
 ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
 GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV

DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

[00394] In **SEQ ID NO:274**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 3 (**SEQ ID NO:172**), and residues 108-214 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00395] A polynucleotide that encodes **SEQ ID NO:274** is (**SEQ ID NO:275**):

```
gacattgtgc tgacccagtc tcacagatcc atgtccacat cagtaggaga
caggggtcaac atcacctgca aggccagtca ggatgtgact actgctgtag
cctgggtatca acaaaaacca gggcaatctc ctaaattact gatTTTTctgg
gcatccaccc ggcacgctgg agtccctgat cgcttcacag gcagtggatc
tgggacagat ttactctca ccatcagcag tgtgcaggct ggagacctgg
cactttatta ctgtcaacaa cattatagca caccgtacac attcggaggg
gggaccaagc tggaaataaa acgtacggtg gctgcaccat cggctcttcac
cttcccgcc tctgatgagc agttgaaatc tggaactgcc tctgttgtgt
gcctgctgaa taacttctat cccagagagg ccaaagtaca gtggaagggtg
gataacgcc tccaatcggg taactcccag gagagtgtca cagagcagga
cagcaaggac agcacctaca gcctcagcag caccctgacg ctgagcaaag
cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt
```

N. EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1

[00396] An alternative EphA2 / CD3 / gpA33 Tri-Specific Binding Molecule was constructed. The molecule was composed of four polypeptide chains and comprises the VL and VH domains of EphA2 mAb 1, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of gpA33 mAb 1. The molecule was designated “**EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1.**” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:276**):

```
DIQMTQTSS LSASLGDRIT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GYTLTYFGGG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PGK
```

[00397] In SEQ ID NO:276, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 1 (SEQ ID NO:153), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 240-245 correspond to the GGCGGG linker (SEQ ID NO:34), residues 246-273 correspond to an E-coil Domain (SEQ ID NO:39), residues 274-276 are the linker GGG, residues 277-286 are the linker DKTHTCPPCP (SEQ ID NO:48), and residues 287-503 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00398] A polynucleotide that encodes SEQ ID NO:276 is (SEQ ID NO:277):

```

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga
cagaatcacc atcagttgca gggcaagtca ggacattagc aattatttaa
actggtatca gcagaaacca gatggaactg ttaaactcct gatctactac
acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc
tggaacagat tattctctca ccattagcaa cctggagcaa gaagatattg
ccacttactt ttgccaacag ggttatacgc tgtacacgtt cggagggggg
accaagctgg aaataaaaagg tggaggatcc ggcggcggag gcgagggtgca
gctggtggag tctgggggag gcttggtcca gcctggaggg tccctgagac
tctcctgtgc agcctctgga ttcaccttca gcacatacgc tatgaattgg
gtccgccagg ctccagggaa ggggctggag tgggttgga ggatcaggtc
caagtacaac aattatgcaa cctactatgc cgactctgtg aagggtagat
tcaccatctc aagagatgat tcaaagaact cactgtatct gcaaataaac
agcctgaaaa ccgaggacac ggccgtgtat tactgtgtga gacacggtaa
cttcggcaat tcttacgtgt cttggtttgc ttattgggga caggggacac
tggtgactgt gtcttccgga ggatgtggcg gtggagaagt ggccgcaactg
gagaaagagg ttgctgcttt ggagaaggag gtcgctgcac ttgaaaagga
ggtcgcagcc ctggagaaaag gcggcgggga caaaactcac acatgcccac
cgtgcccagc acctgaagcc gcggggggac cgtcagtctt cctcttcccc
ccaaaaccca aggacaccct catgatctcc cggaccctg aggtcacatg
cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag ttcaactggt
acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca
ggactggctg aatggcaagg agtacaagtg caaggtctcc aacaaagccc
tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
gaaccacagg tgtacaccct gccccatcc cgggaggaga tgaccaagaa
ccaggtcagc ctgtggtgcc tgggtcaaagg cttctatccc agcgacatcg
ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac
cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgctccgtga
tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct
ccgggtaaa

```

[00399] The amino acid sequence of the second polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:278**):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLKESGPGLV APSQSLSITC TVSGFSLSRV
SVHWVRQPPG KGLEWLGMW GGGSTDYNSA LKSRLSISKD NSKSQVFLKM
NSLQTTDDTAM YYCARKHGNY YTMDYWGQGT SVTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE
```

[00400] In **SEQ ID NO:278**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 1 (**SEQ ID NO:158**), residues 237-242 correspond to the linker GGCGGG (**SEQ ID NO:34**), and residues 243-270 are a K-coil Domain (**SEQ ID NO:40**).

[00401] A polynucleotide that encodes **SEQ ID NO:278** is (**SEQ ID NO:279**):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgacctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcgagg
tggaacaggtg cagctgaagg agtcaggacc tggcctggtg gcaccctcac
agagcctgtc catcacatgc actgtctctg ggttctcatt atccagatat
agtgtacact gggttcgcca gcctccagga aagggctctg agtggctggg
aatgatattg ggtggtggaa gcacagacta taattcagct ctcaaatacca
gactgagtat cagcaaggac aactccaaga gccaaagttt cttaaaaatg
aacagtctgc aaactgatga cacagccatg tactactgtg ccagaaaaca
tggttaactac tatactatgg actactgggg tcaaggaacc tcagtcaccg
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag
```

[00402] The amino acid sequence of the third polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:280**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIY
GNNVYFDVWG QGTTVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTWSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCP CPAPAAAGGP SVFLFPPKPK
```

DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHNRYTQ KSLSLSPGK

[00403] In **SEQ ID NO:280**, amino acid residues 1-119 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (**SEQ ID NO:186**), residues 120-217 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 218-232 correspond to a linker (**SEQ ID NO:209**), and residues 233-449 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00404] A polynucleotide that encodes **SEQ ID NO:280** is (**SEQ ID NO:281**):

caggtccagc tgggtccagag cggggccgaa gtcaaaaaac ccggagcaag
 cgtgaaggct tcctgcaaag catcaggcta tacatttaca ggcagctgga
 tgaactgggt gaggcaggct ccaggacagg gactggagtg gatcgggcgc
 atctaccctg gagacggcga aactaactat aatggaaagt tcaaagaccg
 agtgaccatc acagccgata agtctactag taccgcctac atggagctga
 gctccctgcg gtctgaagat accgccgtct actattgcmc tagaatttac
 ggaaacaatg tctattttga cgtgtggggg caggaacaa ctgtgactgt
 ctctccgccc tccaccaagg gcccatcggc cttccccctg gcaccctcct
 ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac
 tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg ccctgaccag
 cggcgtgcac accttccccg ctgtcctaca gtctcagga ctctactccc
 tcagcagcgt ggtgaccgtg cctccagca gcttgggcac ccagacctac
 atctgcaacg tgaatcacia gccagcaac accaagggtg acaagagagt
 tgagcccaaa tcttgtgaca aaactcacac atgcccaccg tgcccagcac
 ctgaagccgc ggggggaccg tcagtcttcc tcttcccccc aaaaccacaag
 gacaccctca tgatctcccc gacccttgag gtcacatgcm tgggtggtgga
 cgtgagccac gaagaccctg aggtcaagtt caactggtac gtggacggcg
 tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc
 acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa
 tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagcccca
 tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg
 tacaccctgc ccccatcccc ggaggagatg accaagaacc aggtcagcct
 gagttgcgca gtcaaaggct tctatccccg cgacatcgcc gtggagtggg
 agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg
 gactccgacg gctccttctt cctcgtcagc aagctcaccg tggacaagag
 caggtggcag caggggaacg tcttctcatg ctccgtgatg catgaggctc
 tgcacaaccg ctacacgcag aagagcctct ccctgtctcc gggtaaa

[00405] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:282**):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
 SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD

NALQSGNSQE SVTEQDSKDS TYSLSSSTLTL SKADYEKHKV YACEVTHQGL
SSPVTKSFNR GEC

[00406] In **SEQ ID NO:282**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (**SEQ ID NO:181**), and residues 107-213 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00407] A polynucleotide that encodes **SEQ ID NO:282** is (**SEQ ID NO:283**):

```
gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggct ctcaatcagc ttcattgtact
ggatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtgg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaagcg tacgggtggc gcaccatcgg tcttcatctt
cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc
tgctgaataa cttctatccc agagaggcca aagtacagtg gaaggtggat
aacgccctcc aatcgggtaa ctcccaggag agtgtcacag agcaggacag
caaggacagc acctacagcc tcagcagcac cctgacgctg agcaaagcag
actacgagaa acacaaagtc tacgcctgcg aagtcacca tcagggcctg
agctcgcccg tcacaaagag cttcaacagg ggagagtgt
```

O. EphA2 mAb 2 x CD3 mAb 2 x gpA33 mAb 1

[00408] A second alternative EphA2 / CD3 / gpA33 Tri-Specific Binding Molecule was constructed. The molecule was composed of four polypeptide chains and comprises the VL and VH domains of EphA2 mAb 2, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of gpA33 mAb 1. The molecule was designated “**EphA2 mAb 2 x CD3 mAb 2 x gpA33 mAb 1.**” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:284**):

```
DVVMQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
TFGSGTKLEI KGGSGGGGE VQLVESGGGL VQPGGSLRLS CAASGFTFST
YAMNWVRQAP GKGLEWVGRI RSKYNNYATY YADSVKGRFT ISRDDSKNSL
YLQMNSLKTE DTAVYYCVRH GNFGNSYVSW FAYWGQGTLV TVSSGGCGGG
EVAALEKEVA ALEKEVALE KEVAALEKGG GDKTHCPCP PAPEAAGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSREEMT KNQVSLWCLV KGFYPSDIAV EWESNGQPEN
NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK
SLSLSPGK
```

[00409] In SEQ ID NO:284, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 2 (SEQ ID NO:163), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 120-244 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 245-250 correspond to the GGCGGG linker (SEQ ID NO:34), residues 251-278 correspond to an E-coil Domain (SEQ ID NO:39), residues 279-281 are the linker GGG, residues 282-291 are the linker DKHTTCPPCP (SEQ ID NO:48), and residues 292-508 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00410] A polynucleotide that encodes SEQ ID NO:284 is (SEQ ID NO:285):

```

gatgttggtga  tgacccaaac  tccactctcc  ctgcctgtca  gtcttggaga
tcaagcctcc  atctcttgca  gatctagtca  gagccttgta  cacagtagtg
gaaacaccta  ttacattgg  tacctgcaga  agccaggcca  gtctccaaag
ctcctgatct  acaaagtttc  caaccgattt  tctgggggtcc  cagacaggtt
cagtggcagt  ggatcaggga  cagatttcac  actcaagatc  agcagagtgg
aggctgagga  tctgggagtt  tatttctgct  ctcaaagtac  acatgttccc
acgttcggct  cggggacaaa  gttggaaata  aaaggtggag  gatccggcgg
cggaggcgag  gtgcagctgg  tggagtctgg  gggaggcctg  gtccagcctg
gagggtcctc  gagactctcc  tgtgcagcct  ctggattcac  cttcagcaca
tacgctatga  attgggtccg  ccaggctcca  gggaaggggc  tggagtgggt
tggaaggatc  aggtccaagt  acaacaatta  tgcaacctac  tatgccgact
ctgtgaaggg  tagattcacc  atctcaagag  atgattcaaa  gaactcactg
tatctgcaaa  tgaacagcct  gaaaaccgag  gacacggccg  tgtattactg
tgtgagacac  ggtaacttcg  gcaattctta  cgtgtcttgg  tttgcttatt
ggggacaggg  gacactggtg  actgtgtctt  ccggaggatg  tggcgggtgga
gaagtggccg  cactggagaa  agaggttgct  gctttggaga  aggaggtcgc
tgcacttgaa  aaggaggtcg  cagccctgga  gaaaggcggc  ggggacaaaa
ctcacacatg  cccaccgtgc  ccagcacctg  aagccgcggg  gggaccgtca
gtcttcctct  tccccccaaa  acccaaggac  accctcatga  tctcccggac
ccctgaggtc  acatgcgtgg  tgggtggacgt  gagccacgaa  gaccctgagg
tcaagttcaa  ctggtacgtg  gacggcgtgg  aggtgcataa  tgccaagaca
aagccgcggg  aggagcagta  caacagcacg  taccgtgtgg  tcagcgtcct
caccgtcctg  caccaggact  ggctgaatgg  caaggagtac  aagtgcaagg
tctccaacaa  agccctccca  gccccatcg  agaaaaccat  ctccaaagcc
aaagggcagc  cccgagaacc  acaggtgtac  accctgcccc  catcccggga
ggagatgacc  aagaaccagg  tcagcctgtg  gtgcctggtc  aaaggcttct
atcccagcga  catcgccgtg  gagtgggaga  gcaatgggca  gccggagaac
aactacaaga  ccacgcctcc  cgtgctggac  tccgacggct  ccttcttctt
ctacagcaag  ctcaccgtgg  acaagagcag  gtggcagcag  gggaaacgtct
tctcatgctc  cgtgatgcat  gaggtctctg  acaaccacta  cacgcagaag
agcctctccc  tgtctccggg  taaa

```

[00411] The amino acid sequence of the second polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:286**):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQI QLVQSGPELK KPGETVKISC KASGFTFTNY
GMNWVKQAPG KGLKWMGWIN TYIGEPTYAD DFKGRFVFSL ETSASTAYLQ
INNLIKNEDEMA TYFCARELGP YYFDYWGQGT TLTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE
```

[00412] In **SEQ ID NO:286**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 2 (**SEQ ID NO:167**), residues 237-242 correspond to the linker GGCGGG (**SEQ ID NO:34**), and residues 243-270 are a K-coil Domain (**SEQ ID NO:40**).

[00413] A polynucleotide that encodes **SEQ ID NO:286** is (**SEQ ID NO:287**):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcgggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcggagg
tggaacagatc cagttggtgc agtctggacc tgagctgaag aagcctggag
agacagtcaa gatctcctgc aaggcttctg ggtttacctt cacaaactat
ggaatgaact ggggtgaagca ggctccagga aagggtttta agtggtatggg
ctggataaac acctatattg gagagccgac atatgctgat gacttcaagg
gacggtttgt cttctctttg gaaacctctg ccagcactgc ctatttgcag
atcaacaacc tcaaaaatga ggacatggcc acatatttct gtgcaagaga
actgggacca tactactttg actactgggg ccaaggcacc actctcacag
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag
```

[00414] The amino acid sequence of the third polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:288**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIY
GNNVYFDVWG QGTTVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTWSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCP CPAPAAAGGP SVFLFPPKPK
```

DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHNRYTQ KSLSLSPGK

[00415] In **SEQ ID NO:288**, amino acid residues 1-119 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (**SEQ ID NO:186**), residues 120-217 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 218-232 correspond to a linker (**SEQ ID NO:209**), and residues 233-449 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00416] A polynucleotide that encodes **SEQ ID NO:288** is (**SEQ ID NO:289**):

caggtccagc tgggtccagag cggggccgaa gtcaaaaaac ccggagcaag
 cgtgaaggct tcctgcaaag catcaggcta tacatttaca ggcagctgga
 tgaactgggt gaggcaggct ccaggacagg gactggagtg gatcgggcgc
 atctaccctg gagacggcga aactaactat aatggaaagt tcaaagaccg
 agtgaccatc acagccgata agtctactag taccgcctac atggagctga
 gctccctgcg gtctgaagat accgccgtct actattgcmc tagaatttac
 ggaaacaatg tctattttga cgtgtggggg cagggaacaa ctgtgactgt
 ctctccgccc tccaccaagg gcccatcggc cttccccctg gcaccctcct
 ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac
 tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg ccctgaccag
 cggcgtgcac accttccccg ctgtcctaca gtctcagga ctctactccc
 tcagcagcgt ggtgaccgtg cctccagca gcttgggcac ccagacctac
 atctgcaacg tgaatcacia gccagcaac accaagggtg acaagagagt
 tgagcccaaa tcttgtgaca aaactcacac atgcccaccg tgcccagcac
 ctgaagccgc ggggggaccg tcagtcttcc tcttcccccc aaaacccaag
 gacaccctca tgatctcccc gacccttgag gtcacatgcm tgggtggtgga
 cgtgagccac gaagaccctg aggtcaagtt caactggtac gtggacggcg
 tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc
 acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa
 tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagcccca
 tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg
 tacaccctgc ccccatcccc ggaggagatg accaagaacc aggtcagcct
 gagttgcgca gtcaaaggct tctatccccg cgacatcgcc gtggagtggg
 agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg
 gactccgacg gctccttctt cctcgtcagc aagctcaccg tggacaagag
 caggtggcag caggggaacg tcttctcatg ctccgtgatg catgaggctc
 tgcacaaccg ctacacgcag aagagcctct cctgtctctc gggtaaa

[00417] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 2 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:290**):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
 SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD

NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
SSPVTKSFNR GEC

[00418] In **SEQ ID NO:290**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (**SEQ ID NO:181**), and residues 107-213 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00419] A polynucleotide that encodes **SEQ ID NO:290** is (**SEQ ID NO:291**):

```
gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggct ctcaatcagc ttcattgtact
ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtgg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaagcg tacgggtggc gcaccatcgg tcttcatctt
cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc
tgctgaataa cttctatccc agagaggcca aagtacagtg gaaggtggat
aacgccctcc aatcgggtaa ctcccaggag agtgtcacag agcaggacag
caaggacagc acctacagcc tcagcagcac cctgacgctg agcaaagcag
actacgagaa acacaaagtc tacgcctgcg aagtcacca tcagggcctg
agctcgcccc tcacaaagag cttcaacagg ggagagtgt
```

P. EphA2 mAb 3 x CD3 mAb 2 x gpA33 mAb 1

[00420] A third alternative EphA2 / CD3 / gpA33 Tri-Specific Binding Molecule was constructed. The molecule was composed of four polypeptide chains and comprises the VL and VH domains of EphA2 mAb 3, the VL and VH domains of antibody CD3 mAb 2 and the VL and VH domains of gpA33 mAb 1. The molecule was designated “**EphA2 mAb 3 x CD3 mAb 2 x gpA33 mAb 1.**” The amino acid sequence of the first polypeptide chain of this Tri-Specific Binding Molecule is (**SEQ ID NO:292**):

```
DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAVAWYQQKP GQSPKLLIFW
ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISR DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLVTVSS GCGGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPCPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNATKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK
```

[00421] In SEQ ID NO:292, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of EphA2 mAb 3 (SEQ ID NO:172), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 116-240 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 241-246 correspond to the GGCGGG linker (SEQ ID NO:34), residues 247-274 correspond to an E-coil Domain (SEQ ID NO:39), residues 275-277 are the linker GGG, residues 278-287 are the linker DKHTTCPPCP (SEQ ID NO:48), and residues 288-504 are the “knob-bearing” CH2-CH3 Domain (SEQ ID NO:52).

[00422] A polynucleotide that encodes SEQ ID NO:292 is (SEQ ID NO:293):

```

gacattgtgc  tgacccagtc  tcacagatcc  atgtccacat  cagtaggaga
caggggtcaac  atcacctgca  aggccagtca  ggatgtgact  actgctgtag
cctgggtatca  acaaaaacca  gggcaatctc  ctaaattact  gattttctgg
gcatccaccc  ggcacgctgg  agtccctgat  cgcttcacag  gcagtggatc
tgggacagat  ttactctca  ccatcagcag  tgtgcaggct  ggagacctgg
cactttatta  ctgtcaacaa  cattatagca  caccgtacac  attcggaggg
gggaccaagc  tggaaataaa  aggtggagga  tccggcgggc  gaggcgaggt
gcagctggtg  gagtctgggg  gaggcctggg  ccagcctgga  gggtccttga
gactctcctg  tgcagcctct  ggattcacct  tcagcacata  cgctatgaat
tgggtccgcc  aggtctccagg  gaaggggctg  gagtggggtg  gaaggatcag
gtccaagtac  aacaattatg  caacctacta  tgccgactct  gtgaagggtg
gattcaccat  ctcaagagat  gattcaaaga  actcactgta  tctgcaaagt
aacagcctga  aaaccgagga  cacggccgtg  tattactgtg  tgagacacgg
taacttcggc  aattcttacg  tgtcttggtt  tgcttattgg  ggacagggga
cactggtgac  tgtgtcttcc  ggaggatgtg  gcggtggaga  agtggccgca
ctggagaaag  aggttgctgc  tttggagaag  gaggtcgctg  cacttgaaaa
ggaggtcgca  gccctggaga  aaggcggcgg  ggacaaaact  cacacatgcc
caccgtgccc  agcacctgaa  gccgcggggg  gaccgtcagt  cttcctcttc
ccccaaaac  ccaaggacac  cctcatgata  tcccggaccc  ctgagggtcac
atgcgtggtg  gtggacgtga  gccacgaaga  ccctgaggtc  aagttcaact
ggtacgtgga  cggcgtggag  gtgcataatg  ccaagacaaa  gccgcgggag
gagcagtaca  acagcacgta  ccgtgtggtc  agcgtcctca  ccgtcctgca
ccaggactgg  ctgaatggca  aggagtacaa  gtgcaaggtc  tccaacaaag
ccctcccagc  ccccatcgag  aaaaccatct  ccaaagccaa  agggcagccc
cgagaaccac  aggtgtacac  cctgccccca  tcccgggagg  agatgaccaa
gaaccaggtc  agcctgtggt  gcctgtgcaa  aggcttctat  cccagcgaca
tcgccgtgga  gtgggagagc  aatgggcagc  cggagaacaa  ctacaagacc
acgcctcccg  tgctggactc  cgacggctcc  ttcttctctc  acagcaagct
caccgtggac  aagagcaggt  ggcagcaggg  gaacgtcttc  tcatgctccg
tgatgcatga  ggctctgcac  aaccactaca  cgcagaagag  cctctccctg
tctccgggta  aa

```

[00423] The amino acid sequence of the second polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:294**):

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGEV QLVESGGGSV KPGGSLKLSC AASGFTFTDH
YMYWVRQTPE KRLEWVATIS DGSFSTSYDP SVKGRFTISR DIAKNNLYLQ
MSSLKSEDTA MYYCTRDESD RPFPHYWGQGT LVTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE
```

[00424] In **SEQ ID NO:294**, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 3 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 correspond to the amino acid sequence of the VH Domain of EphA2 mAb 3 (**SEQ ID NO:177**), residues 237-242 correspond to the linker GGCGGG (**SEQ ID NO:34**), and residues 243-270 are a K-coil Domain (**SEQ ID NO:40**).

[00425] A polynucleotide that encodes **SEQ ID NO:294** is (**SEQ ID NO:295**):

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgatc
gggggtacaa acaaaagggc tccttgacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggggggtggat ccggcgagg
tgagaaagtg cagctggtgg agtctggggg aggctcagtg aagcctggag
ggtccctgaa actctcctgt gcagcctctg gattcacttt cactgaccat
tacatgtatt gggttcgcca gactccggaa aagaggctgg agtgggtcgc
aaccattagt gatggcggtg gtttcacctc ctatccagac agtgtgaagg
ggcgattcac catctccaga gacattgcca agaacaacct gtacctcaa
atgagcagtc tgaagtctga ggacacagcc atgtattact gtacaagaga
tgagagcgat aggccgttct cttactgggg ccaagggact ctggctactg
tctcctccgg aggatgtggc ggtggaaaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag
```

[00426] The amino acid sequence of the third polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:296**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIY
GNNVYFDVWG QGTTVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTWSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCP CPAPAAAGGP SVFLFPPKPK
```

DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHNRYTQ KSLSLSPGK

[00427] In **SEQ ID NO:296**, amino acid residues 1-119 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (**SEQ ID NO:186**), residues 120-217 correspond to a modified CH1 Domain (**SEQ ID NO:208**), residues 218-232 correspond to a linker (**SEQ ID NO:209**), and residues 233-449 correspond to the “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:53**).

[00428] A polynucleotide that encodes **SEQ ID NO:296** is (**SEQ ID NO:297**):

caggtccagc tgggtccagag cggggccgaa gtcaaaaaaac ccggagcaag
 cgtgaaggct tcctgcaaag catcaggcta tacatttaca ggcagctgga
 tgaactgggt gaggcaggct ccaggacagg gactggagtg gatcgggcgc
 atctaccctg gagacggcga aactaactat aatggaaagt tcaaagaccg
 agtgaccatc acagccgata agtctactag taccgcctac atggagctga
 gctccctgcg gtctgaagat accgccgtct actattgcmc tagaatttac
 ggaaacaatg tctattttga cgtgtggggg cagggaacaa ctgtgactgt
 ctctccgccc tccaccaagg gcccatcggc cttccccctg gcaccctcct
 ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac
 tacttccccg aaccggtgac ggtgtcgtgg aactcaggcg ccctgaccag
 cggcgtgcac accttccccg ctgtcctaca gtctcagga ctctactccc
 tcagcagcgt ggtgaccgtg ccctccagca gcttgggcac ccagacctac
 atctgcaacg tgaatcacia gccccagcaac accaagggtg acaagagagt
 tgagcccaaa tcttgtgaca aaactcacac atgcccaccg tgcccagcac
 ctgaagccgc ggggggaccg tcagtcttcc tcttcccccc aaaacccaag
 gacaccctca tgatctcccc gacccttgag gtcacatgcm tgggtgggtgga
 cgtgagccac gaagaccctg aggtcaagtt caactgggtac gtggacggcg
 tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc
 acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa
 tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagccccc
 tcgagaaaac catctccaaa gccaaagggc agccccgaga accacagggtg
 tacaccctgc ccccatcccc ggaggagatg accaagaacc aggtcagcct
 gagttgcgca gtcaaaggct tctatccccg cgacatcgcc gtggagtggg
 agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg
 gactccgacg gctccttctt cctcgtcagc aagctcaccg tggacaagag
 caggtggcag caggggaacg tcttctcatg ctccgtgatg catgaggctc
 tgcacaaccg ctacacgcag aagagcctct ccctgtctcc gggtaaa

[00429] The amino acid sequence of the fourth polypeptide chain of EphA2 mAb 3 x CD3 mAb 2 x gpA33 mAb 1 is (**SEQ ID NO:298**):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
 SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD

NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
SSPVTKSFNR GEC

[00430] In **SEQ ID NO:298**, amino acid residues 1-106 correspond to the amino acid sequence of the VL Domain of gpA33 mAb 1 (**SEQ ID NO:181**), and residues 107-213 correspond to the CL Kappa Domain (**SEQ ID NO:210**).

[00431] A polynucleotide that encodes **SEQ ID NO:298** is (**SEQ ID NO:299**):

```
gacattcagc tgactcagtc cccctctttt ctgtccgcat ccgtcggaga
tcgagtgact attacttgct ctgctaggct ctcaatcagc ttcattgtact
ggtatcagca gaagcccggc aaagcaccta agctgctgat ctacgacaca
agcaacctgg cctccggggg gccatctcgg ttctctggca gtgggtcagg
aactgagttt accctgacaa ttagctccct ggaggctgaa gatgccgcta
cctactattg ccagcagtgg agcagctatc ctctgacctt cggacagggg
actaaactgg aaatcaagcg tacgggtggc gcaccatcgg tcttcatctt
cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc
tgctgaataa cttctatccc agagaggcca aagtacagtg gaagggtgat
aacgccctcc aatcgggtaa ctcccaggag agtgtcacag agcaggacag
caaggacagc acctacagcc tcagcagcac cctgacgctg agcaaagcag
actacgagaa acacaaagtc tacgcctgcg aagtcacca tcagggcctg
agctcgcccc tcacaaagag cttcaacagg ggagagtgt
```

IV. Reference Antibodies and Diabodies

[00432] In order to assist in the characterization of the Tri-Specific Binding Molecules of the present invention, the following reference diabodies were constructed.

Q. DR5 mAb 1 x CD3 mAb 2 Diabody

[00433] An exemplary bi-specific diabody composed of two polypeptide chains was constructed having the VL and VH domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH domains of CD3 mAb 2. The diabody was designated “**DR5 mAb 1 x CD3 mAb 2 diabody**.” The amino acid sequence of the first polypeptide chain of this diabody is (**SEQ ID NO:140**):

```
DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KGGSGGGGGE VQLVESGGGL VQPGGSLRLS CAASGFTFST
YAMNWVRQAP GKGLEWVGRI RSKYNNYATY YADSVKGRFT ISRDDSKNSL
YLQMNSLKTE DTAVYYCVRH GNFGNSYVSW FAYWGQGTLV TVSSASTKGE
VAACEKEVAA LEKEVAALEK EVAALEK
```

[00434] In SEQ ID NO:140, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (SEQ ID NO:3), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 120-244 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 245-249 correspond to the ASTKG linker (SEQ ID NO:47) and residues 250-277 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41). A polynucleotide that encodes SEQ ID NO:140 is SEQ ID NO:141:

```

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcactggtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtgagg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaaggaggcg gatccggcgg
cggaggcgag gtgcagctgg tggagtctgg gggaggcttg gtccagcctg
gagggtcctt gagactctcc tgtgcagcct ctggattcac cttcagcaca
tacgctatga attgggtccg ccaggctcca gggaaggggc tggagtgggt
tggaaggatc aggtccaagt acaacaatta tgcaacctac tatgccgact
ctgtgaaggg tagattcacc atctcaagag atgattcaaa gaactcactg
tatctgcaaa tgaacagcct gaaaaccgag gacacggccg tgtattactg
tgtgagacac ggtaacttcg gcaattctta cgtgtcttgg tttgcttatt
ggggacaggg gacactggtg actgtgtctt ccgcctccac caagggcgaa
gtggccgcac gtgagaaaga ggttgctgct ttggagaagg aggtcgctgc
acttgaagg gaggtcgcag ccctggagaa a

```

[00435] The amino acid sequence of the second polypeptide chain of the DR5 mAb 1 x CD3 mAb 2 diabody is (SEQ ID NO:142):

```

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTLKTLVLG GGGSGGGGEV KFLESGGGLV QPGGSLKLSC VASGFDFSRY
WMSWVRQAPG KGLEWIGEIN PDSNTINYTP SLKDKFIISR DNAKNTLYLQ
MTKVRSEDTA LYYCTRRAYY GNPWFAYWG QGTLVTVSAA STKGKVAACK
EKVAALKEKV AALKEKVAAL KE

```

[00436] In SEQ ID NO:142, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-239 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8), except that the C-terminal serine residue of SEQ ID NO:8 has been replaced with an alanine residue), residues 240-244 correspond to an

ASTKG linker (SEQ ID NO:47), and residues 245-272 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42). A polynucleotide that encodes SEQ ID NO:142 is SEQ ID NO:143:

```
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac
tgtgacctg acatgcagat ccagcacagg cgcagtgacc acatctaact
acgccaattg ggtgcagcag aagccaggac aggcaccaag gggcctgac
gggggtacaa acaaaagggc tccctggacc cctgcacggt tttctggaag
tctgctgggc ggaaaggccg ctctgactat taccggggca caggccgagg
acgaagccga ttactattgt gctctgtggg atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga ggtggtggat ccggcggcgg
aggcgagggt aagtttctcg agtctggagg tggcctgggt cagcctggag
gatccctgaa actctcctgt gtagcctcag gattcgattt tagtagatac
tggtatgagtt ggggtccggca ggctccaggg aaagggctag aatggattgg
agaaattaat ccagatagca atacgataaa ctatacgcca tctctaaagg
ataaattcat catctccaga gacaacgcca aaaatacgct gtatctgcaa
atgaccaaag tgagatctga ggacacagcc ctttattatt gtacaagaag
ggcctactat ggtaaccggc cctgggtttgc ttactggggc caagggactc
tggtcactgt ctctgcagcc tccaccaagg gcaaagtggc cgcagtgaag
gagaaagttg ctgctttgaa agagaaggtc gccgcactta aggaaaagg
cgcagccctg aaagag
```

R. DR5 mAb 2 x CD3 mAb 2 Diabody

[00437] An exemplary bi-specific diabody composed of two polypeptide chains was constructed having the VL and VH domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH domains of CD3 mAb 2. The diabody was designated “**DR5 mAb 2 x CD3 mAb 2 diabody**.” The amino acid sequence of the first polypeptide chain of this diabody is (SEQ ID NO:144):

```
DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAWAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGIRISKY NNYATYYADS VKGRFTISR DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGT LVT VSS ASTKGEVAAC
EKEVA ALEKE VAALEKEVAA LEK
```

[00438] In SEQ ID NO:144, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (SEQ ID NO:13), residues 108-115 correspond to intervening spacer peptide (Linker 1) (SEQ ID NO:33), residues 116-240 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 241-245 correspond to an ASTKG linker (SEQ ID NO:47) and residues 246-273 correspond to a cysteine-containing E-

coil Domain (SEQ ID NO:41). A polynucleotide that encodes SEQ ID NO:144 is SEQ ID NO:145:

```

gacattgtga  tgacccagtc  tcacaaattc  atgtccactt  cagtaggaga
caggggtcagc  atcacctgca  aggccagtc  ggatgtgaat  actgctgtag
cctgggtatca  acaaaaacca  gggcaatctc  ctaaactact  gatttactgg
gcattccaccc  ggcacactgg  agtccctgat  cgcttcacag  gcagtggatc
tgggacagat  tatacactca  ccatcaaaag  tgtgcaggct  gaagacctga
cactttatta  ctgtcagcaa  cactatatca  ctccgtggac  gttcggtgga
ggcaccaagc  tggaaatcaa  aggaggcgga  tccggcggcg  gaggcgaggt
gcagctggtg  gagtctgggg  gaggcttgg  ccagcctgga  gggctccctga
gactctcctg  tgcagcctct  ggattcacct  tcagcacata  cgctatgaat
tgggtccgcc  aggtccagg  gaaggggctg  gagtgggttg  gaaggatcag
gtccaagtac  aacaattatg  caacctacta  tgccgactct  gtgaagggtg
gattcaccat  ctcaagagat  gattcaaaga  actcactgta  tctgcaaagt
aacagcctga  aaaccgagga  cacggccgtg  tattactgtg  tgagacacgg
taacttcggc  aattcttacg  tgtcttggtt  tgcttattgg  ggacagggga
cactggtgac  tgtgtcttcc  gcctccacca  agggcggaag  ggccgcatgt
gagaaagagg  ttgctgcttt  ggagaaggag  gtcgctgcac  ttgaaaagga
ggtcgcagcc  ctggagaaa

```

[00439] The amino acid sequence of the second polypeptide chain of the DR5 mAb 2 x CD3 mAb 2 diabody is (SEQ ID NO:146):

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QAVVTQEPSL  TVSPGGTVTL  TCRSSTGAVT  TSNYANWVQQ  KPGQAPRGLI
GGTNKRAPWT  PARFSGSLLG  GKAALTITGA  QAEDEADYYC  ALWYSNLWVF
GGGTKLTVLG  GGGSGGGGKV  QLQQSGAELV  KPGASVKLSC  KASGYTFTEY
ILHWVKQKSG  QGLEWIGWIFY  PGNNNIKYNE  KFKDKATLTA  DKSSSTVYME
LSRLTSEDSA  VYFCARHEQG  PGYFDYWGG  TTLTVSSAST  KGKVAACKEK
VAALKEKVAA  LKEKVAALKE

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[00440] In SEQ ID NO:146, amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-237 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (SEQ ID NO:18), residues 238-242 correspond to an ASTKG linker (SEQ ID NO:47), and residues 243-270 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42). A polynucleotide that encodes SEQ ID NO:146 is SEQ ID NO:147:

```

caggctgtgg  tgactcagga  gccttcactg  accgtgtccc  caggcggaac
tgtgaccctg  acatgcagat  ccagcacagg  cgcagtgacc  acatctaact
acgccaatgt  ggtgcagcag  aagccaggac  aggaccaag  gggcctgac
gggggtacaa  acaaaagggc  tccttgagac  cctgcacggg  tttctggaag
tctgctgggc  ggaaaggccg  ctctgactat  taccggggca  caggccgagg
acgaagccga  ttactattgt  gctctgtggt  atagcaatct  gtgggtgttc

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gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggcgg
aggcaaggtc cagctgcagc agtctggagc tgaactggtg aaaccgcggg
catcagtga gctgtcctgc aaggcttctg ggtacacctt cactgagtat
atthttacact gggtaaagca gaagtctgga cagggtcttg agtggattgg
gtgggttttat cctggaaata ataataataa gtacaatgag aaattcaagg
acaaggccac actgactgcg gacaaatcct ccagcacagt ctatatggaa
cttagtagat tgacatctga agactctgcg gtctatthtct gtgcaagaca
cgaacaagga ccaggttact ttgactactg gggccaaggc accactctca
cagtctcctc cgctccacc aaggggcaaag tggccgcatg taaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgacgc
cctgaaagag

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S. DR5 mAb 3 x CD3 mAb 2 Diabody

[00441] An exemplary bi-specific diabody composed of two polypeptide chains was constructed having the VL and VH domains of anti-human DR5 antibody DR5 mAb 3 and the VL and VH domains of CD3 mAb 2. The amino acid sequence of the first polypeptide chain of the diabody had the sequence (SEQ ID NO:148) (CDR residues are shown underlined):

```

SELTQDPAVS VALGQTVRIT CSGDSLRSYY ASWYQQKPGQ APVLVIYGAN
NRPSGIPDRF SGSSSGNTAS LTITGAQAED EADYYCNSAD SSGNHVVFGG
GTKLTVLGGG GSGGGGEVQL VESGGGLVQP GGSLRLSCAA SGFTFSTYAM
NWVRQAPGKG LEWVGRIRSK YNNYATYYAD SVKGRRFTISR DDSKNSLYLQ
MNSLKTEDTA VYYCVRHGNF GNSYVSWFAY WGQGTTLVTVS SASTKGEVAA
CEKEVAALEK EVAALEKEVA ALEK

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[00442] In SEQ ID NO:148, amino acid residues 1-108 correspond to the VL Domain of DR5 mAb 3 (SEQ ID NO:54), residues 109-116 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 117-241 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 242-246 correspond to an ASTKG linker (SEQ ID NO:47), and residues 247-275 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42).

[00443] The amino acid sequence of the second polypeptide chain of the diabody had the sequence (SEQ ID NO:149) (CDR residues are shown underlined):

```

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGEV QLVQSGGGVE RGGSLRLSC AASGFTFDDY
AMSWVRQAPG KGLEWVSGIN WQGGSTGYAD SVKGRRVTISR DNAKNSLYLQ

```

MNSLRAEDTA VYYCAKILGA GRGWYFDYWG KGTTVTVSSA STKGKVAACK
EKVAALKEKV AALKEKVAAL KE

[00444] In SEQ ID NO:149, amino acid residues 1-110 correspond to the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-239 correspond to the amino acid sequence of the VH Domain of DR5 mAb 3 (SEQ ID NO:58), residues 240-244 correspond to an ASTKG linker (SEQ ID NO:47), and residues 245-272 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42).

T. DR5 mAb 4 x CD3 mAb 2 Diabody

[00445] An exemplary bi-specific diabody composed of two polypeptide chains was constructed having the VL and VH domains of anti-human DR5 antibody DR5 mAb 4 and the VL and VH domains of CD3 mAb 2. The amino acid sequence of the first polypeptide chain of the diabody had the sequence (SEQ ID NO:150) (CDR residues are shown underlined):

EIVLTQSPGT LSLSPGERAT LSCRASQGIS RSYLAWYQQK PGQAPSLLIY
GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QFGSSPWTFG
QGTVKEIKGG GSGGGGEVQL VESGGGLVQP GGSRLRLSCAA SGFTFSTYAM
NWVRQAPGKG LEWVGRIRSK YNNYATYYAD SVKGRFTISR DDSKNSLYLQ
MNSLKTEDTA VYYCVRHGNF GNSYVSWFAY WGQGTSLVTVS SASTKGEVAA
CEKEVAALEK EVAALEKEVA ALEK

[00446] In SEQ ID NO:150, amino acid residues 1-108 correspond to the VL Domain of DR5 mAb 4 (SEQ ID NO:62), residues 109-116 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 117-241 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 242-246 correspond to an ASTKG linker (SEQ ID NO:47), and residues 247-275 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41).

[00447] The amino acid sequence of the second polypeptide chain of the diabody had the sequence (SEQ ID NO:151) (CDR residues are shown underlined):

QAVVTQEPSTL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLQESGPGLV KPSQTLSTLC TVSGGSISSG

DYFWSWIRQL PGKGLEWIGH IHNSGTTYYN PSLKSRVTTIS VDTSKKQFSL
 RLSSVTAADT AVYYCARDDRG GDYYYGMDVW GGGTTVTVSS ASTKGKVAAC
 KEKVAALKEK VAALKEKVAA LKE

[00448] In SEQ ID NO:151, amino acid residues 1-110 correspond to the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-240 correspond to the amino acid sequence of the VH Domain of DR5 mAb 4 (SEQ ID NO:66), residues 241-245 correspond to an ASTKG linker (SEQ ID NO:47), and residues 246-273 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42).

U. Reference gpA33 x CD3 mAb 2 Diabody

[00449] To further exemplify the bi-specific Tri-Specific Binding Molecules of the present invention, a diabody composed of two polypeptide chains was constructed using the VL and VH domains of gpA33 mAb 1 and CD3 mAb 2. The amino acid sequence of the first polypeptide chain of the diabody had the sequence (SEQ ID NO:316) (CDR residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTSSLEAE DAATYYCQQW SSYPLTFGQG
 TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTTYAMNW
 VRQAPGKGLE WVGRIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
 SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSA STKGEVAACE
 KEVAALEKEV AALEKEVAAL EK

[00450] In SEQ ID NO:316, amino acid residues 1-106 correspond to the VL Domain of gpA33 mAb 1 (SEQ ID NO:181), residues 107-114 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 115-239 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (SEQ ID NO:112), residues 240-244 correspond to an ASTKG linker (SEQ ID NO:47), and residues 245-272 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41).

[00451] The amino acid sequence of the second polypeptide chain of the diabody had the sequence (SEQ ID NO:317) (CDR residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GTNKRAPWPT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF

GGGTLTLVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTGS
WMNWVRQAPG QGLEWIGRIY PGDGETNYNG KFKDRVTTTA DKSTSTAYME
 LSSLRSEDTA VYYCARIIYGN NVYFDVWGQG TTVTSSAST KGKVAACKEK
 VAALKEKVAA LKEKVAALKE

[00452] In SEQ ID NO:317, amino acid residues 1-110 correspond to the VL Domain of CD3 mAb 2 (SEQ ID NO:104), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 119-237 correspond to the amino acid sequence of the VH Domain of gpA33 mAb 1 (SEQ ID NO:186), residues 238-242 correspond to an ASTKG linker (SEQ ID NO:47), and residues 243-270 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42).

V. Reference Anti-Fluorescein Antibody

[00453] The anti-fluorescein antibody 4-4-20 (Gruber, M. et al. (1994) "Efficient Tumor Cell Lysis Mediated By A Bi-specific Single Chain Antibody Expressed In Escherichia coli," J. Immunol. 152(11):5368-5374; Bedzyk, W.D. et al. (1989) "Comparison Of Variable Region Primary Structures Within An Anti-Fluorescein Idiotypic Family," J. Biol. Chem. 264(3): 1565-1569) was used in control diabodies. The amino acid sequences of the variable light and variable heavy Domains of anti-fluorescein antibody 4-4-20 are as follows:

[00454] Amino Acid Sequence Of The Variable Light Chain Domain Of Anti-Fluorescein Antibody 4-4-20 (SEQ ID NO:138) (CDR residues are underlined):

DVVMQTTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK
 VLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
WTFGGGTKLE IK

[00455] Amino Acid Sequence Of The Variable Heavy Chain Domain Of Anti-Fluorescein Antibody 4-4-20 (SEQ ID NO:139) (CDR residues are underlined):

EVKLDETGGG LVQPGRPMKL SCVASGFTFS DYWMNWVRQS PEKGLEWVAQ
IRNKPYNYET YYSDSVKGRF TISRDDSKSS VYLQMNNLRV EDMGIYYCTG
SYYGMDYWGQ GTSVTSS

V. Methods of Production

[00456] The Tri-Specific Binding Molecules of the present invention can be created from the polynucleotides and/or sequences of antibodies that are

immunospecific for DR5, a desired Cancer Antigen, and a desired Effector Cell by methods known in the art, for example, synthetically or recombinantly. One method of producing such peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well-known to those skilled in the art (see, *e.g.*, Kelley, R. F. *et al.* (1990) In: GENETIC ENGINEERING PRINCIPLES AND METHODS, Setlow, J.K. Ed., Plenum Press, N.Y., vol. 12, pp 1-19; Stewart, J.M *et al.* (1984) SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL; see also United States Patents Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[00457] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) “*Solid Phase Synthesis*,” Science 232(4748):341-347; Houghten, R.A. (1985) “*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*,” Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) “*Solid-Phase Synthesis In The Twenty-First Century*,” Mini Rev. Med. Chem. 6(1):3-10).

[00458] In yet another alternative, suitable antibodies having one or more of the CDRs of a desired anti-DR5 antibody, anti-Cancer Antigen antibody or anti-Effector Cell antibody may be obtained through the use of commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XENOMOUSE™ (Abgenix, Inc., Fremont, CA) and HUMAB-MOUSE® and TC MOUSE™ (both from Medarex, Inc., Princeton, NJ).

[00459] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*,

CHO cells). Another method that may be employed is to express the antibody sequence in plants {e.g., tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) “*Production Of Antibodies And Antibody Fragments In Plants*,” Vaccine 19:2756; Lonberg, N. *et al.* (1995) “*Human Antibodies From Transgenic Mice*,” Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) “*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*,” J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, etc. are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) “*Making Antibodies By Phage Display Technology*,” Annu. Rev. Immunol. 12:433-455).

[00460] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well-known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[00461] An alternative method of cloning the protein of interest is by “panning” using purified DR5, and/or a desired Cancer Antigen, and/or a molecule expressed on the surface of a desired Effector Cell (or portions of any such molecules), for cells expressing an antibody or protein of interest that possesses one or more CDRs so as to be capable of binding to DR5, or such desired Cancer Antigen or Effector Cell molecule. The “panning” procedure may be conducted by obtaining a cDNA library from tissues or cells that express DR5, overexpressing the cDNAs in a second cell type, and screening the transfected cells of the second cell type for a specific binding to DR5 in the presence or absence of a known antibody that is capable of binding to such molecule (*e.g.*, DR5 mAb 1 or DR5 mAb 2 in the case of panning for new anti-DR5 antibodies, *etc.*). Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by “panning” can be found in the art (see, for example, Aruffo, A. *et al.* (1987) “*Molecular Cloning Of A CD28 cDNA By A High-Efficiency COS Cell Expression System*,” Proc. Natl. Acad. Sci. (U.S.A.)

84:8573-8577 and Stephan, J. *et al.* (1999) "*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation,*" *Endocrinol.* 140:5841-5854).

[00462] Vectors containing polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00463] Any host cells capable of overexpressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5-fold higher, more preferably 10-fold higher, even more preferably 20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to DR5 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

[00464] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an anti-DR5 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

[00465] The invention includes modifications to any such antibodies (or to any of their polypeptide fragments that bind to DR5, the Cancer Antigen or the effector cell, as the case may be) and the agonists, antagonists, and modulators of such molecules, including functionally equivalent antibodies and fusion polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, i.e., the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00466] The invention encompasses fusion proteins comprising one or more of the polypeptides of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin

constant region. In another embodiment, the fusion polypeptide contains a light chain variable region and a heavy chain variable region of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to DR5, a Cancer Antigen, or an effector cell (as the case may be) and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

VI. Uses of the Trispecific Binding Molecules of the Present Invention

[00467] The Tri-Specific Binding Molecules of the present invention provide a general therapy for cancer. The cancers that may be treated by such molecules include cancers characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocyoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine

cancer. The Tri-Specific Binding Molecules of the present invention may be used in the treatment of colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer.

[00468] The Tri-Specific Binding Molecules of the present invention augment the cancer therapy provided by an antibody directed to a Cancer Antigen that is characteristic of cells of a target tumor by being additionally able to bind to DR5 molecules arrayed on the surface of such tumor cells. The utility of the invention is particularly seen in circumstances in which the density of the Cancer Antigen is low, or when the binding kinetics of the anti-Cancer Antigen antibody is suboptimal (or insufficient) to promote a clinically sufficient therapeutic response. In such cases, the ability of the molecules of the present invention to bind both the Cancer Antigen and DR5 of the tumor cells provides enhanced binding (via avidity) that is sufficient to promote a clinically sufficient therapeutic response. Additionally, by also possessing a Binding Domain capable of binding to a molecule on the surface of an immune system effector cell, the Tri-Specific Binding Molecules of the present invention permit the co-localization of such immune system cells to the tumor cells, thereby promoting a cytotoxic response against the tumor cells via redirected killing.

[00469] As shown in **Table 2**, Tri-Specific Binding Molecules of the present invention that possess particular combinations of Cancer Antigen-Binding Domains have preferred utility in the treatment of specific cancers.

Table 2		
Cancer Antigen-Binding Domains		Preferred Utility
gpA33	DR5	Treatment Of Colorectal Cancer
gpA33	EphA2	
gpA33	B7-H3	
gpA33	BST2	
5T4	EphA2	Broadly Applicable To Treatment Of Many Types Of Cancers
5T4	CEACAM5	
5T4	B7-H3	
5T4	DR5	
B7-H3	CEACAM5	
B7-H3	CEACAM6	

Table 2		
Cancer Antigen-Binding Domains		Preferred Utility
B7-H3	IL1R α 2	Glioblastoma, Melanoma
EphA2	IL1R α 2	
EphA2	DR5	Broadly Applicable To Treatment Of Many Types Of Cancers
EphA2	CEACAM5	
EphA2	CEACAM6	
ITGB6	B7-H3	
ITGB6	DR5	
ITGB6	BST2	
BST2	CEACAM5	
BST2	EGFR	

[00470] In addition to their utility in therapy, the Tri-Specific Binding Molecules of the present invention may be detectably labeled and used in the diagnosis of cancer or in the imaging of tumors and tumor cells.

VII. Pharmaceutical Compositions

[00471] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the Tri-Specific Binding Molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the Tri-Specific Binding Molecules of the present invention and a pharmaceutically acceptable carrier. The invention particularly encompasses such pharmaceutical compositions in which the Tri-Specific Binding Molecule has a DR5-Binding Domain of:

- (1) a DR5 mAb 1 antibody;
- (2) a DR5 mAb 2 antibody;
- (3) a DR5 mAb 3 antibody;
- (4) a DR5 mAb 4 antibody;
- (5) a DR5 mAb 5 antibody;
- (6) a DR5 mAb 6 antibody;
- (7) a DR5 mAb 7 antibody; or

(8) a DR5 mAb 8 antibody
(or a humanized derivative of any such antibodies).

[00472] The invention further particularly encompasses such pharmaceutical compositions in which the Tri-Specific Binding Molecule has a Cancer Antigen-Binding Domain that:

- (A) binds to an epitope of EphA2, especially wherein the Tri-Specific Binding Molecule has a Cancer Antigen-Binding Domain of EphA2 mAb 1, EphA2 mAb 2 or EphA2 mAb 3, or a humanized or chimeric variant thereof; or
- (B) binds to an epitope of gpA33, especially wherein the Tri-Specific Binding Molecule has a Cancer Antigen-Binding Domain of gpA33 mAb 1, or a humanized or chimeric variant thereof; or
- (C) binds to an epitope of Her2, especially wherein the Tri-Specific Binding Molecule has a Cancer Antigen-Binding Domain of Her2 mAb 1 or trastuzumab, or a humanized or chimeric variant thereof; or
- (D) binds to an epitope of B7-H3, especially wherein the Tri-Specific Binding Molecule has a Cancer Antigen-Binding Domain of B7-H3 mAb 1, B7-H3 mAb 2, or B7-H3 mAb 3, or a humanized or chimeric variant thereof.

[00473] The invention further particularly encompasses such pharmaceutical compositions in which the Tri-Specific Binding Molecule has an Effector Cell-Binding Domain that binds to CD2, CD3, CD17, CD20, CD22, CD32B, CD64, BCR/CD79, the T cell Receptor or the NKG2D Receptor. The invention further particularly encompasses such pharmaceutical compositions in which the Tri-Specific Binding Molecule has an Effector Cell-Binding Domain of antibody: Lo-CD2a, CD3 mAb 2, OKT3, 3G8, A9, HD37, rituximab, epratuzumab, CD32B mAb 1, CD64 mAb 1, CD79 mAb 1, BMA 031, KYK-1.0, or KYK-2.0.

[00474] The invention specifically contemplates Tri-Specific Binding Molecules, pharmaceutical compositions that comprise such binding molecule and uses of such Tri-Specific Binding Molecules, in which:

- (1) the DR5 Binding Domain is a DR5 binding domain of any anti-DR5 antibody;
 - (2) the Cancer Binding Domain is any of the Cancer Antigens disclosed herein;
- and
- (3) the Effector Cell-Binding Domains binds to any of CD2, CD3, CD17, CD20, CD22, CD32B, CD64, BCR/CD79, the T cell Receptor or the NKG2D Receptor.

[00475] The invention further specifically contemplates Tri-Specific Binding Molecules, pharmaceutical compositions that comprise such binding molecule and uses of such Tri-Specific Binding Molecules, in which:

- (1) the DR5 Binding Domain is a DR5 binding domain of any anti-DR5 antibody;
 - (2) the Cancer Binding Domain is any of: EphA1, gpA33, Her2, or B7-H3;
- and
- (3) the Effector Cell-Binding Domains binds to any of CD2, CD3, CD17, CD20, CD22, CD32B, CD64, BCR/CD79, the T cell Receptor or the NKG2D Receptor.

[00476] The invention particularly contemplates each of the Tri-Specific Binding Molecules, as well as pharmaceutical compositions that comprise such binding molecule and uses of such Tri-Specific Binding Molecules, in which:

- (1) the DR5 Binding Domain is a DR5 binding domain of any of: a DR5 mAb 1 antibody, a DR5 mAb 2 antibody, a DR5 mAb 3 antibody, a DR5 mAb 4 antibody, a DR5 mAb 5 antibody, a DR5 mAb 6 antibody, a DR5 mAb 7 antibody, or a DR5 mAb 8 antibody;
 - (2) the Cancer Antigen-Binding Domain is a binding domain of any of: EphA2 mAb 1, EphA2 mAb 2, EphA2 mAb 3, gpA33 mAb 1, Her2 mAb 1, trastuzumab, B7-H3 mAb 1, B7-H3 mAb 2, or B7-H3 mAb 3;
- and
- (3) the Effector Cell-Binding Domain is a binding domain of any of: Lo-CD2a, CD3 mAb 2, OKT3, 3G8, A9, HD37, rituximab, epratuzumab,

CD32B mAb 1, CD64 mAb 1, CD79 mAb 1, BMA 031, KYK-1.0, or KYK-2.0.

As 8 anti-DR5 Binding Domain antibodies, 9 anti-Cancer Antigen-Binding Domain antibodies and 14 anti-Effector Cell-Binding Domain antibodies are listed, such specific contemplation encompasses all (8 x 9 x 14 =) 1,008 combinations of such binding domains.

[00477] The invention also encompasses such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor-specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00478] In a specific embodiment, the term “**pharmaceutically acceptable**” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “**carrier**” refers to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00479] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry

lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00480] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00481] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a Tri-Specific Binding Molecule of the present invention (and more preferably, any of the specific binding molecules discussed or exemplified above). Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00482] The present invention provides kits that can be used in the above methods. A kit can comprise any of the Tri-Specific Binding Molecules of the present invention. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain embodiments, the other

prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

VIII. Methods of Administration

[00483] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or a conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or a conjugated molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00484] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) "*Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System*," J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00485] Methods of administering a molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the Tri-Specific Binding Molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition,

pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00486] The invention also provides that the Tri-Specific Binding Molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the Tri-Specific Binding Molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 µg, more preferably at least 10 µg, at least 15 µg, at least 25 µg, at least 50 µg, at least 100 µg, or at least 200 µg.

[00487] The lyophilized Tri-Specific Binding Molecules of the present invention should be stored at between 2 and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such Tri-Specific Binding Molecules when provided in liquid form are supplied in a hermetically sealed container in which the molecules are present at a concentration of least 1 µg/ml, more preferably at least 2.5 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 50 µg/ml, or at least 100 µg/ml.

[00488] The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the

seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00489] For the Tri-Specific Binding Molecules monovalent diabodies encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. The dosage administered is typically from at least about 0.3 ng/kg per day to about 0.9 ng/kg per day, from at least about 1 ng/kg per day to about 3 ng/kg per day, from at least about 3 ng/kg per day to about 9 ng/kg per day, from at least about 10 ng/kg per day to about 30 ng/kg per day, from at least about 30 ng/kg per day to about 90 ng/kg per day, from at least about 100 ng/kg per day to about 300 ng/kg per day, from at least about 200 ng/kg per day to about 600 ng/kg per day, from at least about 300 ng/kg per day to about 900 ng/kg per day, from at least about 400 ng/kg per day to about 800 ng/kg per day, from at least about 500 ng/kg per day to about 1000 ng/kg per day, from at least about 600 ng/kg per day to about 1000 ng/kg per day, from at least about 700 ng/kg per day to about 1000 ng/kg per day, from at least about 800 ng/kg per day to about 1000 ng/kg per day, from at least about 900 ng/kg per day to about 1000 ng/kg per day, or at least about 1,000 ng/kg per day.

[00490] In another embodiment, the patient is administered a treatment regimen comprising one or more doses of such prophylactically or therapeutically effective amount of a Tri-Specific Binding Molecule of the present invention, wherein the treatment regimen is administered over 2 days, 3 days, 4 days, 5 days, 6 days or 7 days. In certain embodiments, the treatment regimen comprises intermittently administering doses of the prophylactically or therapeutically effective amount of the Tri-Specific Binding Molecules of the present invention (for example, administering a dose on day 1, day 2, day 3 and day 4 of a given week and not administering doses of the prophylactically or therapeutically effective amount of the Tri-Specific Binding Molecule

[00491] Especially encompassed is the administration of such Tri-Specific Binding Molecules that comprise any of the specific combinations of DR5 Binding Domains,

Cancer Antigen-Binding Domains and Effector Cell-Binding Domains discussed above, on day 5, day 6 and day 7 of the same week). Typically, there are 1, 2, 3, 4, 5 or more courses of treatment. Each course may be the same regimen or a different regimen.

[00492] In another embodiment, the administered dose escalates over the first quarter, first half or first two-thirds or three-quarters of the regimen(s) (*e.g.*, over the first, second, or third regimens of a 4 course treatment) until the daily prophylactically or therapeutically effective amount of the Tri-Specific Binding Molecule is achieved. **Table 3** provides 5 examples of different dosing regimens described above for a typical course of treatment.

Table 3						
Regimen	Day	Diabody Dosage (ng diabody per kg subject weight per day)				
1	1, 2, 3, 4	100	100	100	100	100
	5, 6, 7	none	none	none	none	none
2	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
3	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
4	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none

[00493] The dosage and frequency of administration of a Tri-Specific Binding Molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00494] The dosage of a Tri-Specific Binding Molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00495] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of

the invention, care must be taken to use materials to which the molecule does not absorb.

[00496] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) "New Methods Of Drug Delivery," Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327).

[00497] The compositions of the invention can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more of the Tri-Specific Binding Molecule(s) of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) "Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) "Antibody Mediated Lung Targeting Of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) "Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) "Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (*See* Langer, *supra*; Sefton, (1987) "Implantable Pumps," CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) "Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis," Surgery 88:507-516; and Saudek *et al.* (1989) "A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery," N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled-release of the molecules (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND

PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) "*Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate*," Science 228:190-192; During *et al.* (1989) "*Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization*," Ann. Neurol. 25:351-356; Howard *et al.* (1989) "*Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits*," J. Neurosurg. 7(1):105-112; U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. A controlled-release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). Polymeric compositions useful as controlled-release implants can be used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. A non-polymeric sustained delivery system can be used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

[00498] Controlled-release systems are discussed in the review by Langer (1990, "*New Methods Of Drug Delivery*," Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et*

al. (1996) "Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) "Antibody Mediated Lung Targeting Of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) "Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) "Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00499] Where the composition of the invention is a nucleic acid encoding a Tri-Specific Binding Molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded Tri-Specific Binding Molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (See U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.* (1991) "Antennapedia Homeobox Peptide Regulates Neural Morphogenesis," Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00500] Treatment of a subject with a therapeutically or prophylactically effective amount of a Tri-Specific Binding Molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The pharmaceutical compositions of the invention can be administered once a day, twice a day, or three times a day. Alternatively, the pharmaceutical compositions can be administered once a week, twice a week, once every two weeks, once a month, once

every six weeks, once every two months, twice a year or once per year. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

Examples:

[00501] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

W. Example 1: Characterization of Anti-Human DR5 Monoclonal Antibodies DR5 mAb 1 and DR5 mAb 2

[00502] Two monoclonal antibodies were isolated as being capable of immunospecifically binding to human DR5, and accorded the designations “DR5 mAb 1” and “DR5 mAb 2”. As discussed above, the CDRs of these antibodies were found to differ. In order to determine whether the antibodies bound to different DR5 epitopes, a human DR5-Fc fusion protein was prepared and was coated to an immobilized surface. DR5 mAb 1 (1 µg/mL) was biotinylated and incubated with either a control IgG or with DR5 mAb 2 (10 µg/mL), and the ability of the IgG or DR5 mAb 2 antibody to compete for binding (to human DR5-Fc fusion protein) with DR5 mAb 1 was assessed by measuring the amount of immobilized biotinylated antibody. Additionally, the ability of the IgG or DR5 mAb 1 antibody to compete for binding with biotinylated DR5 mAb 2 was assessed. The results of this experiment are shown in **Table 4**.

Table 4				
1 µg/mL DR5-Fc Fusion coat		10 µg/mL Competitor mAb		
		mIgG	DR5 mAb 1	DR5 mAb 2
1 µg/mL biotinylated DR5 mAb	DR5 mAb 1	2.162	self	0.826
	DR5 mAb 2	2.102	2.377	self

[00503] The results of this experiment indicate that the biotinylated antibody was capable of binding to the DR5 protein even in the presence of excess amounts of the non-biotinylated antibody. Thus, the results show that DR5 mAb 1 and DR5 mAb 2 bind to different epitopes of DR5.

[00504] In order to further characterize the DR5 mAb 1 and DR mAb 2 antibodies, their ability to block binding between DR5 and the TRAIL ligand as assessed. Thus, biotinylated DR5 mAb 1, biotinylated DR5 mAb 2 or biotinylated DR5-Fc fusion (each at 2 µg/mL) were separately incubated with immobilized DR5-Fc fusion (1 µg/mL) in the presence of either buffer or histidine tagged TRAIL (20 µg/mL). The amount of immobilized biotinylated antibody was assessed. The results of this experiment are shown in **Table 5**.

Table 5			
2 µg/mL Biotinylated DR5 mAb	1 µg/mL DR5-Fc fusion coat		1 µg/mL TRAIL- His coat
	20 µg/mL TRAIL- His	Buffer	
DR5 mAb 1	1.939	2.118	0.007
DR5 mAb 2	2.052	2.052	0.008
DR5-Fc fusion	--	--	0.288

[00505] The results show that the amount of DR5 mAb 1 or DR5 mAb 2 bound to the immobilized DR5-Fc was not affected by the presence of the histidine tagged TRAIL, thus indicating that neither DR5 mAb 1 nor DR5 mAb 2 block the TRAIL ligand binding site of DR5. Additionally, neither antibody was capable of binding to the histidine tagged TRAIL ligand.

X. Example 2: Species Specificity of Anti-Human DR5 Monoclonal Antibodies DR5 mAb 1 and DR5 mAb 2

[00506] In order to assess the species specificity of anti-human DR5 monoclonal antibodies DR5 mAb 1 and DR5 mAb 2, the ability of the antibodies to bind to human DR5 was compared with their ability to bind cynomolgus monkey (*Macaca fascicularis*) DR5. The results of this experiment are shown in **Figure 6**. The results show that both antibodies are capable of binding to cynomolgus monkey DR5, but that they each exhibit higher binding affinity for human DR5.

[00507] The kinetics of binding was investigated using Biacore Analysis, as shown in **Figure 7**. Bi-specific DR5 x CD3 diabodies were incubated with His-tagged DR5 and the kinetics of binding was determined via Biacore analysis. The diabodies employed were DR5 mAb 1 x CD3 mAb 2 (**Figure 7, Panels A and E**), DR5 mAb 2 x CD3 mAb 2 (**Figure 7, Panels B and F**), DR5 mAb 3 x CD3 mAb 2 (**Figure 7, Panels C and G**), and DR5 mAb 4 x CD3 mAb 2 (**Figure 7, Panels D and H**). **Figure 7, Panels A-D** show the results for human DR5. **Figure 7, Panels E-H** show the results for cynomolgus monkey DR5. The calculated k_a , k_d and K_D are presented in **Table 6**.

Table 6						
Anti-DR Antibody	Human			Cynomolgus Monkey		
	k_a	k_d	K_D (nM)	k_a	k_d	K_D (nM)
DR mAb 1	8.5×10^6	1.2×10^{-3}	0.14	4.0×10^6	1.3×10^{-1}	32.5
DR mAb 2	3.4×10^5	2.1×10^{-4}	0.62	2.4×10^5	1.0×10^{-4}	0.42
DR mAb 3	4.2×10^6	3.7×10^{-2}	8.8	3.3×10^6	4.4×10^{-2}	13.3
DR mAb 4	5.4×10^6	1.7×10^{-2}	3.2	2.5×10^6	4.1×10^{-2}	16.4

[00508] The results demonstrate that DR5 mAb 1 and DR5 mAb 2 exhibit altered kinetics of binding relative to reference antibodies DR5 mAb 3 and DR5 mAb 4.

Y. Example 3: Unexpected Superiority of DR5 mAb 1 and DR5 mAb 2

[00509] The ability of DR5-binding molecules DR5 mAb 1 and DR5 mAb 2 of the present invention to mediate cytotoxicity was compared with that of the reference anti-DR5 antibodies: DR5 mAb 3 and DR5 mAb 4. In order to make such a comparison, a bi-specific DR5 x CD3 diabody containing the VL and VH Domains of these antibodies and the VL and VH Domains of CD3 mAb 2 were prepared. The prepared diabodies were: DR5 mAb 1 x CD3 mAb 2; DR5 mAb 2 x CD3 mAb 2; DR5 mAb 3 x CD3 mAb 2; and DR5 mAb 4 x CD3 mAb 2.

[00510] The employed control diabody contained the VL and VH domains of anti-fluorescein antibody 4-4-20 (respectively, **SEQ ID NOs:138 and 139**) and the VL and VH domains of CD3 mAb 2 (respectively, **SEQ ID NOs:102 and 108**), and was designated as the anti-fluorescein x anti-CD3 control diabody “**4-4-20 x CD3 mAb 2.**” The diabody was composed of two polypeptide chains. The first polypeptide

chain of the diabody had the amino acid sequence (**SEQ ID NO:300**) (CDRs are shown in underline):

DVVMQTQPFSL LPVSLGDQAS ISC**RSSQSLV** **HSNGNTYLRW** YLQKPGQSPK
 VLIY**KVSNRF** **SGVPDRFSGS** GSGTDFTLKI SRVEAEDLGV YFC**SQSTHVP**
WTFGGGTKLE IKGGSGGGG EVQLVESGGG LVQPGGSLRL SCAASGFTFN
TYAMNWVRQA PGKGLEWVAR **IRSKYNNYAT** **YYADSVKDRF** TISRDDSKNS
 LYLQMNSLKT EDTAVYYCVR **HGNFGNSYVS** **WFAY**WGQGTL VTVSSGGCGG
 GEVAALEKEV AALEKEVAAL EKEVAALEK

[00511] In **SEQ ID NO:300**, amino acid residues 1-112 correspond to the VL Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:138**), residues 113-120 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 121-245 correspond to the VH Domain of CD3 mAb 2 (**SEQ ID NO:108**), residues 246-251 are a cysteine-containing spacer peptide (GGCGGG) (**SEQ ID NO:34**), and residues 252-280 correspond to an E-coil Domain (**SEQ ID NO:39**).

[00512] The second polypeptide chain of the diabody had the amino acid sequence (**SEQ ID NO:301**) (CDRs are shown in underline):

QAVVTQEPSL TVSPGGTVTL TCR**RSSTGAVT** **TSNYAN**WVQQ KPGQAPRGLI
 G**GTNKR**APWT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWVF**
 GGGTKLTVLG GGGSGGGGEV KLDETGGGLV QPGRPMKLSC **VASGFTFSDY**
WMNWVRQSPE KGLEWVA**QIR** **NKPYNYETYY** **SDSVKGRFTI** SRDDSKSSVY
 LQMNNLRVED MGIYYCTG**SY** **YGM**DYWGQGT SVTVSSGGCG GGVAAALKEK
 VAALKEKVAA LKEKVAALKE

[00513] In **SEQ ID NO:301**, amino acid residues 1-110 correspond to the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 correspond to the VH Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:139**), residues 237-242 are a cysteine-containing spacer peptide (GGCGGG) (**SEQ ID NO:34**), and residues 243-270 correspond to a K-coil Domain (**SEQ ID NO:40**).

[00514] Target tumor cells were incubated with one of these diabodies or with the control diabody (4-4-20 x CD3 mAb 2) in the presence of peripheral blood mononuclear cells (PBMC) and A549 adenocarcinomic human alveolar basal epithelial cells for 24 hours at an effector to target cell ratio of 20:1. The percentage cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells.

[00515] The results of this investigation are shown in **Figure 8**. Similar results were obtained using SKMES human lung cancer cells, DU145 human prostate cancer cells, A375 human malignant melanoma cells, SKBR3 human HER2-overexpressing breast carcinoma cells, and JIMT human breast carcinoma cells. The results indicate that the VL and VH domains of DR5 mAb 1 and DR5 mAb 2 are significantly and unexpectedly more potent in inducing cytotoxicity than those of the reference DR5 mAbs.

Z. Example 4: The Trispecific Binding Molecules Mediate Coordinated and Simultaneous Binding to Target Cells

[00516] The ability of Tri-Specific Binding Molecules of the present invention to bind to target cells was investigated. The employed trispecific molecules were: EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1; EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1; and gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1. As shown in **Figure 9A**, those Tri-Specific Binding Molecules that comprise an EphA2 Cancer Antigen-Binding Domain were found to be capable of binding to EphA2-expressing CHO target cells. As shown in **Figure 9B**, those Tri-Specific Binding Molecules that comprise a DR5 Cancer Antigen-Binding Domain were found to be capable of binding to DR5-expressing CHO target cells. As shown in **Figure 9C**, those Tri-Specific Binding Molecules that comprise an EphA2 Cancer Antigen-Binding *or* a DR5 Cancer Binding Domain were found to be capable of binding to DU145 cells. DU145 cells are a human prostate cell line that express both EphA2 and DR5, but not gpA33. The above-described reference gpA33 mAb 1 x CD3 mAb 2 diabody was used as a control.

[00517] Significantly, the data show that when *both* of the two Cancer Antigen-Binding Domains of a Tri-Specific Binding Molecule of the present invention are able to bind to a target cell, such dual binding is associated with a synergistic (*e.g.*, a 5-25 fold) enhancement in target binding.

AA. Example 5: The Trispecific Binding Molecules Mediate Cytotoxicity of Bound Target Cells

[00518] The ability of Tri-Specific Binding Molecules of the present invention to mediate the cytotoxicity of bound target cells in the presence of cytotoxic lymphocytes was investigated. The employed trispecific molecules were: EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1; EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1; and gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1. The above-described reference gpA33 mAb 1 x CD3 mAb 2 diabody and the 4-4-20 x CD3 mAb 2 diabody were used as controls.

[00519] As shown in **Figure 10A**, those Tri-Specific Binding Molecules that comprise an EphA2 Cancer Antigen-Binding Domain, and thus were able to bind to bind to EphA2-expressing CHO cells, were able to mediate the cytotoxicity of such cells in the presence of the cytotoxic lymphocytes. As shown in **Figure 10B**, those Tri-Specific Binding Molecules that comprise a DR5 Cancer Antigen-Binding Domain, and thus were able to bind to bind to DR5-expressing CHO cells, were found to be capable of mediating cytotoxicity of DR5-expressing CHO target cells in the presence of the cytotoxic lymphocytes.

[00520] As shown in **Figure 10C**, those Tri-Specific Binding Molecules that comprise an EphA2 Cancer Antigen-Binding *or* a DR5 Cancer Binding Domain, and thus are capable of binding to DU145 cells, were able to mediate the cytotoxicity of such cells in the presence of the cytotoxic lymphocytes. Significantly, the data show that when *both* of the two Cancer Antigen-Binding Domains of a Tri-Specific Binding Molecule of the present invention are able to bind to a target cell, such dual binding is associated with a synergistic enhancement in target binding. Thus, EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1, which is capable of binding to *both* EphA2 *and* DR5, mediated substantially greater cytotoxicity than EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 or gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1, which were capable of binding to only EphA2 or DR5 molecules of the DU146 cells (since such cells lack gpA33).

[00521] In this regard, at approximately the EC50 of EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1, no cytotoxic lymphocyte response is seen for either EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 or gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1. At approximately the EC90 of EphA2 mAb 1 x CD3 mAb 2 x DR5 mAb 1, EphA2 mAb 1 x CD3 mAb 2 x gpA33 mAb 1 shows only an EC15, and gpA33 mAb 1 x CD3 mAb 2 x DR5 mAb 1 shows no cytotoxic lymphocyte response at all.

[00522] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

Claim 1. A Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:

(I) four different polypeptide chains covalently complexed together;

(II) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(IV) a Fc Domain, wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—
(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain);
or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—
(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—
(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—
(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3
Domain);

(E) a fourth polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{III} Domain)—(Cysteine-Containing Domain);

(F) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III: and

(G) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

Claim 2. The Tri-Specific Binding Molecule of claim 1, wherein the Cysteine-Containing Domain of the third polypeptide chain is chosen from a CH1 Domain and/or a Hinge Domain and the Cysteine-Containing Domain of the fourth polypeptide chain is a CL Domain.

Claim 3. The Tri-Specific Binding Molecule of claim 1 or claim 2, wherein (i) the first polypeptide chain and the second polypeptide chain are covalently bonded to one another; (ii) the first polypeptide chain and the third polypeptide chain are covalently bonded to one another; and (iii) the third polypeptide chain and the fourth polypeptide chain are covalently bonded to one another.

Claim 4. A Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:

(I) three different polypeptide chains covalently complexed together;

(II) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(IV) a Fc Domain; wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—
(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain);
or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—
(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—
(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—
(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, in the N-terminus to C-terminus direction:

(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain); or

(Cysteine-Containing Domain 1)—(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain 2)—
(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III; and

(F) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

Claim 5. The Tri-Specific Binding Molecule of claim 4, wherein the Cysteine-Containing Domain 1 of the third polypeptide chain is a CL Domain and the Cysteine-Containing Domain 2 of the third polypeptide chain is chosen from a CH1 Domain and/or a Hinge Domain.

Claim 6. The Tri-Specific Binding Molecule of any one of claims 1-5, wherein:
the Linker 2 comprises a cysteine residue, or
the Heterodimer-Promoting Domain comprises a cysteine residue, or
the Linker 2 comprises a cysteine residue and the Heterodimer-Promoting Domain comprises a cysteine residue.

Claim 7. The Tri-Specific Binding Molecule of any one of claims 1-6, wherein:
the CH2-CH3 Domain of the first polypeptide chain is a knob-bearing CH2-CH3 Domain and the CH2-CH3 Domain of the third polypeptide chain is a hole-bearing CH2-CH3 Domain; or
the CH2-CH3 Domain of the third polypeptide chain is a knob-bearing CH2-CH3 Domain and the CH2-CH3 Domain of the first polypeptide chain is a hole-bearing CH2-CH3 Domain.

Claim 8. The Tri-Specific Binding Molecule of any one of claims 1-7, wherein the CH2-CH3 Domain of the first polypeptide chain and the third polypeptide chain independently comprises:

(A) one substitution selected from the group consisting of:

F243L, R292P, Y300L, V305I, and P396L;

(B) two substitutions selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) three substitutions selected from the group consisting of:

- (1) F243L, R292P and Y300L;
- (2) F243L, R292P and V305I;
- (3) F243L, R292P and P396L; and
- (4) R292P, V305I and P396L;

(D) four substitutions selected from the group consisting of:

- (1) F243L, R292P, Y300L and P396L; and
- (2) F243L, R292P, V305I and P396L; or

(E) five substitutions selected from the group consisting of:

- (1) F243L, R292P, Y300L, V305I and P396L; and
- (2) L235V, F243L, R292P, Y300L and P396L

Claim 9. The Tri-Specific Binding Molecule of any one of claims 1-8, wherein:

the Heterodimer-Promoting Domain on the first polypeptide chain is an E-coil Domain and the Heterodimer-Promoting Domain on the second polypeptide chain is a K-coil Domain; or

the Heterodimer-Promoting Domain on the first polypeptide chain is an K-coil Domain and the Heterodimer-Promoting Domain on the second polypeptide chain is a E-coil Domain.

Claim 10. The Tri-Specific Binding Molecule of any one of claims 1-9, wherein:
the Linker 1 comprises the sequence of SEQ ID NO: 33;

the Linker 2 comprises the sequence of SEQ ID NO: 34 or 47;

the Heterodimer-Promoting Domain comprises the sequence of SEQ ID NO: 35, 36, 37, 38, 39, 40, 41 or 42;

the Linker 3 comprises the sequence of SEQ ID NO: 46, 47, 48, 49, 50, 51, 152, or GCG or GGG;

the Linker 4 comprises the sequence of SEQ ID NO: 50 or 152;

the CH2-CH3 Domain comprises the sequence of SEQ ID NO: 52 or 53;

the CH1-Hinge Domain comprises the sequence of SEQ ID NO: 207, 208 or 209; or

the Cysteine-Containing Domain comprises the sequence of SEQ ID NO: 34, 36, 38, 48, 210 or 211.

Claim 11. The Tri-Specific Binding Molecule of claim 10, wherein the Heterodimer-Promoting Domain of SEQ ID NO: 41 or 42 is present when Linker 2 comprises the sequence of SEQ ID NO: 47, or the Heterodimer-Promoting Domain of SEQ ID NO: 39, 40, 41 or 42 is present when Linker 2 comprises the sequence of SEQ ID NO: 34.

Claim 12. The Tri-Specific Binding Molecule of any one of claims 1-11, wherein:

- (A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the second Cancer Antigen and an epitope of the Effector Cell Antigen;
 - (B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the second Cancer Antigen;
 - (C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of second Cancer Antigen, an epitope of the first Cancer Antigen, and an epitope of the Effector Cell Antigen;
 - (D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the second Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the first Cancer Antigen;
 - (E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of the first Cancer Antigen, and an epitope of the second Cancer Antigen;
- or

- (F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of second Cancer Antigen, and an epitope of the first Cancer Antigen.

Claim 13. The Tri-Specific Binding Molecule of any one of claims 1-12, wherein the Effector Cell Antigen is CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, B cell Receptor (BCR), T cell Receptor (TCR), or NKG2D Receptor.

Claim 14. The Tri-Specific Binding Molecule of any of claims 1-13, wherein:

- (A) the epitope of an Effector Cell Antigen is a CD2 epitope recognized by antibody Lo-CD2a;
- (B) the epitope of an Effector Cell Antigen is a CD3 epitope recognized by antibody OKT3, M291, YTH12.5 or Anti-CD3 mAb 2, which antibody Anti-CD3 mAb 2 comprises SEQ ID NO:104 and SEQ ID NO:108 or 112;
- (C) the epitope of an Effector Cell Antigen is a CD16 epitope recognized by antibody 3G8 or A9;
- (D) the epitope of an Effector Cell Antigen is a CD19 epitope recognized by antibody MD1342, MEDI-551, blinatumomab or HD37;
- (E) the epitope of an Effector Cell Antigen is a CD20 epitope recognized by antibody rituximab, ibritumomab, ofatumumab, and tositumomab;
- (F) the epitope of an Effector Cell Antigen is a CD22 epitope recognized by antibody epratuzumab;
- (G) the epitope of an Effector Cell Antigen is a CD32B epitope recognized by antibody CD32B mAb 1, which antibody CD32B mAb 1 comprises SEQ ID NO:126 and SEQ ID NO:127;
- (H) the epitope of an Effector Cell Antigen is a CD64 epitope recognized by antibody CD64 mAb 1, which CD64 mAb 1 comprises SEQ ID NO:128 and SEQ ID NO:129;

- (I) the epitope of an Effector Cell Antigen is a BCR/CD79 epitope recognized by antibody CD79 mAb 1, which antibody CD79 mAb 1 comprises SEQ ID NO:130 and SEQ ID NO:131;
- (J) the epitope of an Effector Cell Antigen is a TCR epitope recognized by antibody BMA 031;
- (K) the epitope of an Effector Cell Antigen is a NKG2D Receptor epitope recognized by antibody KYK-2.0; or
- (L) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope comprises the six CDRs of SEQ ID NO:102 and 103; 104 and 108; 104 and 112; 114 and 115; 116 and 117; 118 and 119; 120 and 121; 122 and 123; 124 and 125; 126 and 127; 128 and 129; 130 and 131; 132 and 133; 134 and 135; or 136 and 137; or comprises one or more of SEQ ID NO: 102-104, 108, 112, 114 and 115-137.

Claim 15. The Tri-Specific Binding Molecule of any one of claims 1-14, wherein the first Cancer Antigen and the second Cancer Antigen are independently chosen from: colon cancer antigen 19.9; a gastric cancer mucin; antigen 4.2; glycoprotein A33 (gpA33); ADAM-9; gastric cancer antigen AH6; ALCAM; malignant human lymphocyte antigen APO-1; cancer antigen B1; B7-H3; beta-catenin; blood group ALeb/Ley; Burkitt's lymphoma antigen-38.13, colonic adenocarcinoma antigen C14; ovarian carcinoma antigen CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD30 ; CD33; CD36; CD45; CD46; CD52 ; CD79a/CD79b; CD103; CD317 ; CDK4; carcinoembryonic antigen (CEA); CEACAM5; CEACAM6; CO17-1A; CO-43 (blood group Leb); CO-514 (blood group Lea); CTA-1; CTLA4; Cytokeratin 8; antigen D1.1; antigen D156-22; DR5; E1 series (blood group B); EGFR (Epidermal Growth Factor Receptor); Ephrin receptor A2 (EphA2); ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; lung adenocarcinoma

antigen F3; antigen FC10.2; G49, ganglioside GD2; ganglioside GD3; ganglioside GM2; ganglioside GM3; GD2; GD3; GICA 19-9; GM2; gp100; human leukemia T cell antigen Gp37; melanoma antigen gp75; gpA33; HER2 antigen (p185HER2); human milk fat globule antigen (HMFG); human papillomavirus-E6/human papillomavirus-E7; high molecular weight melanoma antigen (HMW-MAA); I antigen (differentiation antigen) I(Ma); Integrin Alpha-V-Beta-6 Integrin β 6 (ITGB6); Interleukin-13; Receptor α 2 (IL13R α 2); JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; human lung carcinoma antigens L6 and L20; LEA; LUCA-2; M1:22:25:8; M18; M39; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; Myl; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; melanoma-associated antigen p97; polymorphic epithelial mucin (PEM); polymorphic epithelial mucin antigen (PEMA); PIPA; prostate-specific antigen (PSA); prostate-specific membrane antigen (PSMA); prostatic acid phosphate; R24; ROR1; sphingolipids; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; T5A7; TAG-72; TL5 (blood group A); TNF- α receptor; TNF- β receptor; TNF- γ receptor; TRA-1-85 (blood group H); Transferrin Receptor; tumor-specific transplantation antigen (TSTA), oncofetal antigen-alpha-fetoprotein (AFP); VEGF, VEGFR; VEP8; VEP9; VIM-D5; and Y hapten, Le^y; and 5T4.

- Claim 16. The Tri-Specific Binding Molecule of claim 15, wherein one of Epitope I, Epitope II or Epitope III is an epitope of a Cancer Antigen and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope comprises the six CDRs of SEQ ID NO: 3 and 8; 13 and 18; 23 and 31; 25 and 31; 27 and 31; 29 and 31; 54 and 58; 62 and 66; 70 and 74; 78 and 82; 86 and 90; 94 and 98; 153 and 158; 163 and 167; 172 and 177; 181 and 186; 191 and 192; 193 and 194; 195 and 196; 197 and 198; 199 and 200; 201 and 202; 203 and 204; 205 and 206; 302 and 303; 304 and 305; 306 and 307; 308 and 309; 310 and 311; 312 and 313; 314 and 315; or 321 and

322; or comprises one or more of SEQ ID NO: 3, 8, 13, 18, 23, 25, 27, 29, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98, 153, 158, 163, 167, 172, 177, 181, 186, 191-206, 302-315, 321 and 322.

- Claim 17. A nucleic acid composition comprising polynucleotides that encode polypeptide chains of the Tri-Specific Binding Molecule of any one of claims 1-16.
- Claim 18. A pharmaceutical composition comprising the Tri-Specific Binding Molecule of any one of claims 1-16, and a pharmaceutically acceptable carrier, excipient or diluent.
- Claim 19. A Tri-Specific Binding Molecule of any one of claims 1-16, or a composition of claim 18, for use as a medicament.
- Claim 20. Use of a Tri-Specific Binding Molecule of any one of claims 1-16 or a composition of claim 18 in the manufacture of a medicament to treat a cancer.
- Claim 21. The use of claim 20, wherein the cancer is characterized by the presence of a cancer cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocyoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a

lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a hematological malignancy, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, or a uterine cancer.

Claim 22. The use of claim 20 or claim 21, wherein the cancer is a colorectal cancer, a hepatocellular carcinoma, a glioma, a kidney cancer, a breast cancer, a multiple myeloma, bladder cancer, a neuroblastoma, a sarcoma, a non-Hodgkin's lymphoma, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphoma (NHL), mantle cell leukemia (MCL), small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.